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MRB3009F

Final Technical Report

to the

Office of Naval Research
and
Advanced Research Projects Agency
ARPA Order No. 299, Amend. 6
Contract Nonr 4511 (00)
Task NR 356-464

CHEMILUMINESCENT SYSTEMS

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CHEMILUMINESCENT SYSTEMS

Monsanto Research Corporation
Boston Laboratory
Everett, Massachusetts 02149
Tel: 617-389-0480

30 November 1966

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FOREWORD

This is the final technical report on contract Nonr-4511(00), "Chemiluminescent Systems," covering work done from 1 June 1964 to 31 May 1966. The project was sponsored by Advanced Research Projects Agency under Order No. 299, Task Nr. 356-464, and was monitored by the Office of Naval Research.

The program was led by Dr. Abraham W. Berger until December 1965, at which time Dr. John S. Driscoll became Project Leader. Contributors to the work included Dr. Morton H. Gollis, who advised on organic preparations, Mr. W.R. Smith, who was responsible for analytical work, Mr. Harry R. DiPietro, who prepared many compounds, and Mr. J.N. Driscoll, research technician

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DEFINITIONS AND ABBREVIATIONS

I	Photocurrent reading of the detecting photomultiplier at a specified time of oxidation. The current is proportional to the chemiluminescence intensity.
I_{max}	Photocurrent reading at the maximum of the chemiluminescence emission curve.
I_o	Photocurrent reading of a blue tritiated phosphor. A Luminous Products Corp., Boston Mass., Model NEP-1 blue phosphor has an emission intensity of 5 microlamberts. The chemiluminescence observed from a system is compared to this standard emitter as a ratio: I_{max}/I_o or I/I_o.
$t_{1/2}$	The time required for the emission intensity to decay to one-half of its maximum value.
Figure of Merit (FM)	The time-integrated light output (figure of merit) is approximated by the product of the maximum intensity and the half life. The analytical expression ($I_{max} \times t_{1/2}$) approximates the area under the chemiluminescence emission curve assuming (1) that the time to reach maximum intensity is small compared to the decay half life, $t_{1/2}$, and (2) that the decay is exponential.
I_L/I_F	Relative current reading expected for emissions from luminol (I_L) and fluorescein (I_F) as detected by an RCA IP 28 photomultiplier (see Appendix III).
L	The light capacity of a chemiluminescent system defined as $L = 4.07 \times 10^4$ QMP, where Q is the quantum yield, M is the concentration of the chemiluminescent compound, and P is the phototropic efficiency.
DEP	Diethylphthlate
DMSO	Dimethylsulfoxide
DMF	N,N-Dimethylformamide
HPT	Hexamethylphosphorictriamide
AAB	o-Acetamidobenzaldehyde
FAP	o-Formidoacetophenone
AAP	o-Acetamidoacetophenone

t-BuOK	Potassium tertiary butoxide
TMAE	Tetrakis dimethylaminoethylene
CrAA	Chromium acetylacetonate
CuAA	Copper acetylacetonate
FeAA	Iron acetylacetonate
DPA	9,10-Diphenylanthracene
ANSA	4-Amino-1-naphthylenesulfonic Acid
BBO	2,5-Dibiphenyloxazole
PPO	2,5-Diphenyloxazole
POPOP	p-Bis[2-(5-phenyloxazolyl)]-benzene
TP	p-Terphenyl

I. INTRODUCTION AND SUMMARY

The objective of this research program was the discovery of bright chemiluminescent reactions suitable for development into illumination and marking devices for field use. Our approach to this problem was divided into three main phases. First, a screening program was conducted to determine promising classes of new chemiluminescent compounds. Second, the three most promising classes were screened more thoroughly. The best system was studied in detail. Third, the possibility of enhanced emission as the result of reaction at a solid-liquid interface was studied.

In preparation for the screening program, a study concerned with the optimization of chemiluminescence reaction variables was undertaken. The nature and purity of the reaction solvent were found to be very important. Non-aqueous solvents were found to possess many advantages. While chemiluminescent compounds gave comparable emission intensities in dimethylsulfoxide (DMSO) and dimethylformamide solutions, the former solvent was found to be the more stable under the reaction conditions employed. Potassium t-butoxide was found to be the most useful basic material in DMSO. The DMSO/t-BuOK/O_2 system was chosen as optimum for the screening work.

In excess of 175 organic compounds were investigated during the general screening program. It may be concluded from the data that chemiluminescence is a very general phenomenon. Almost every compound studied gave at least weak emission.

Three compound classes appeared the most promising. Additional derivations of the acyloins, electronegatively substituted olefins, and the indoles were screened.

The chemiluminescence emission λ_{max} for the benzoin system was found to correspond exactly to the 505 mμ benzil fluorescence emission peak. These data strongly suggest that the first excited singlet state is the emitter in the benzoin chemiluminescent system.

At the end of the secondary screening program, it was apparent that the indole system was the best of the three. A detailed study of the effect of substituents on indole chemiluminescence was carried out. An investigation of 56 indole derivatives showed numerous explainable substituent effects. Of greatest importance was the necessity for a proton on the heterocyclic nitrogen atom, and an alkyl group on the 3-position. Skatole (3-methylindole), a natural product, fulfills these conditions and was found to be the brightest system studied.

The chemiluminescent emitter in the skatole system was unambiguously identified as the excited singlet state of the anion

of ortho formamidoacetophenone (FAP). The agreement between the chemiluminescence spectrum of the skatole system, and the fluorescence emission spectrum of FAP is among the best to be found for any known system. The emitter in the 2,3-dimethyl indole system was shown to have a structure analogous to FAP.

The chemiluminescence efficiency of the skatole system was found to be 0.05-0.1%. The light capacity, at optimum conditions, is 0.16 lumen-hours per liter.

The possibility that solid substrates might enhance chemiluminescent emission through the stabilization of excited states or by the catalysis of the formation of unstable intermediates (e.g. hydroperoxides) was studied. Special cells and photometers were designed and constructed for this purpose. Catalysis materials (Pt, Pd, Ag, charcoal, SiO_2, Al_2O_3, etc.) were bonded to membranes and compared with Teflon as an inert standard. Membrane reflectivity proved to be a critical factor influencing the total light emission. A number of fluorescent energy transfer agents were screened. Fluroescein was found to be the only dye useful in the dimethyl-sulfoxide-potassium t-butoxide system as an energy acceptor.

II. MAXIMIZATION OF CHEMILUMINESCENCE EMISSION PARAMETERS

A. INTRODUCTION

The chemiluminescence efficiency depends on a large number of variables. The influence of reagent concentration, and differences in oxidizing agents, bases, catalysts and solvents are important experimental parameters affecting the efficiency of the chemical reactions leading to electronically excited molecules. It was felt that if any of these critical variables could be optimized, important dividends would be realized in the screening program.

A study of solvent effects on known chemiluminescent systems (luminol, lucigenin, lophine and pyrogallol) offered a promising and potentially useful approach towards enhancement of light yields. Since many of the organic reactants and products investigated were polar, the metastable intermediates involved might also have this characteristic and therefore be especially solvent sensitive.

B. EXPERIMENTAL

All organic solvents were "Spectrograde" (except as noted). Inorganic reacts were all reagent grades. Both were used without further purification. A number of sensitizers ordinarily prepared as high purity components of scintillators were also used without further purification*. Melting point determinations and thin layer chromatographic separations were carried out for the reducing (organic) reactants. The observed and literature data are compared in Table 1.

Table 1

MELTING POINTS OBSERVED FOR REACTANTS

Compound	Literature, °C	Observed, °C
Luminol	323-333	312-314 (dec)
Lucigenin	410[a]	400 (dec)
Pyrogallol	132-133	117-122

[a] Tetrahydrate

* Obtained from Pilot Chemical Company, Watertown, Massachusetts

The observed values did not agree with reported melting points too well. However, since no impurity separations were observed for any of the above materials in T.L.C. analysis, the compounds were used as supplied for this phase of the work, except for the luminol and lophine standards, which were prepared from freshly recrystallized materials (ref. 34).

C. SOLVENT CHEMILUMINESCENCE

The solvents utilized in lucigenein, lophine and pyrogallol oxidation were tested for chemiluminescent emission. Emission flashes were observed upon mixing. This solvent emission was several orders of magnitude lower than that observed when the organic materials were present.

Tetrahydrofuran was found to be a relatively intense emitter compared with other unsensitized solvents. Preliminary results with acetone has shown that the weak emission in the pure solvent may be increased by several orders of magnitude by addition of scintilliators such as POPOP, tetraphenylbutadiene or p-terphenyl. These sensitizers produce a visible pulse in the acetone-peroxide system.

D. ALKALINE PEROXIDE OXIDATIONS OF LUCIGENIN

1. General Solvent Effects

The work of Weber (ref. 1, 2) concerning solvent effects in the alkaline peroxide oxidation of lucigenin was extended. In Figure 1, the overall light yield, $I_{max} \times t_{1/2}$, is plotted against the dielectric constants of several aqueous-organic solvent mixtures. All mixtures contained 18.7 volume percent of the organic component.

With DMSO and acetone, compounds containing base sensitive hydrogen atoms, no significant emission changes were observed relative to a completely aqueous system. In aprotic solvents, however, [dioxance, pyridine, and dimethylformamide (DMF)] light outputs were found to be an order of magnitude greater. The emission from the tetrahydrofuran (THF) mixture is two orders of magnitude greater than from the pure aqueous system.

The pronounced influence of the organic solvents is more clearly demonstrated in Figure 2 where the peak intensity is shown as a function of half-life in mixed solvents. However, the lack of adequate reproducibility of the data with respect to the total and relative intensities limits the interpretation of the experimental results.

The large effects observed in the THF solvent system has been subsequently attributed to light emission from the reaction

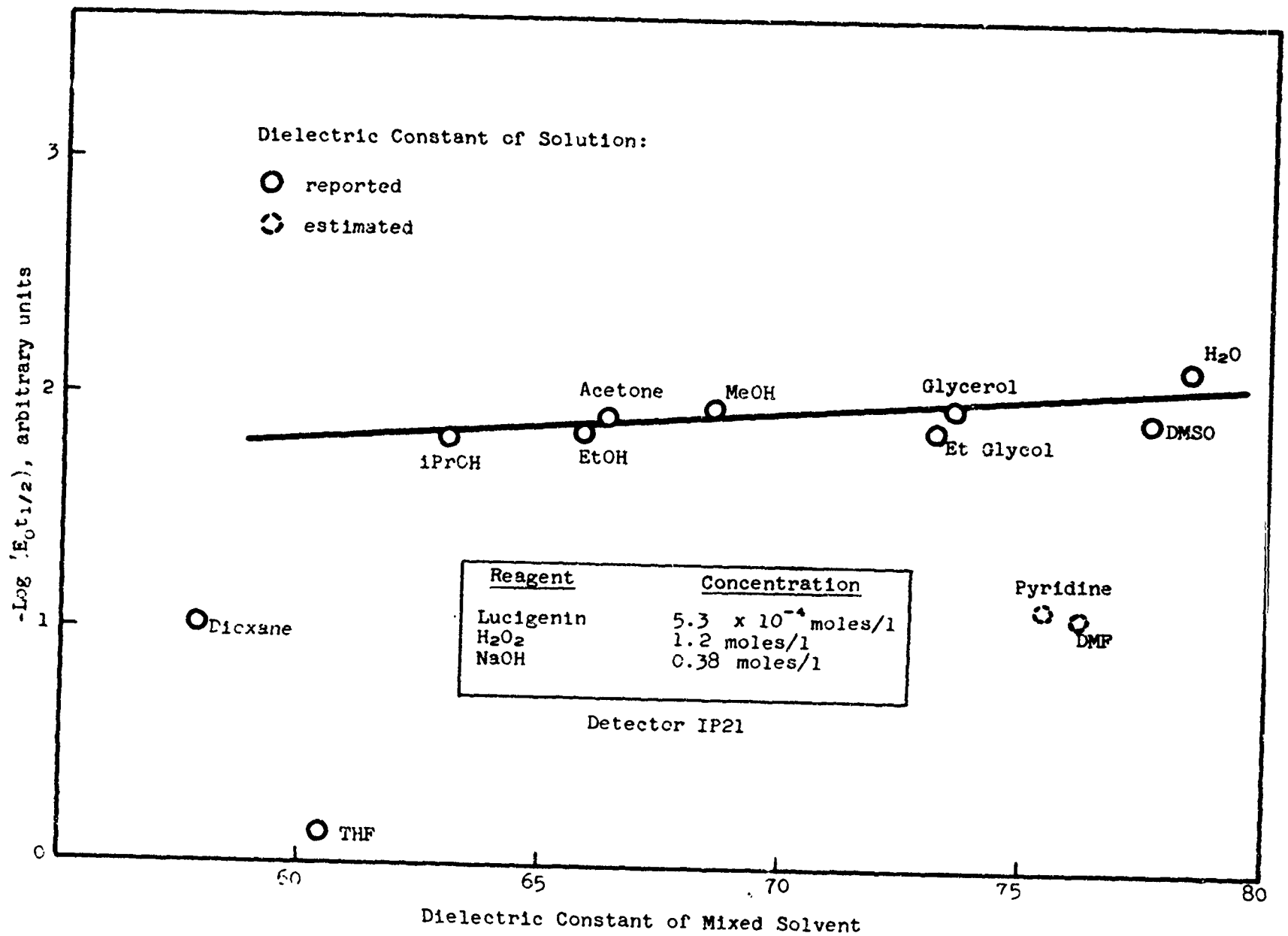


Figure 1. Peak Intensity x Half-life as a Function of the Dielectric Constant of Mixed Solvents for Lucigenin Oxidation (at 18.7 vol-% organic)

with the THF itself or an impurity. Comparison of the solvent oxidation results of Table 2 with the data reported in Figures 1 and 2 suggest that a correlation may exist between solvent oxidation effects and mixed solvent emission.

2. Dioxane Concentration Effects

Figure 3 shows the results of dioxane concentration effects upon the chemiluminescence of lucigenin. Logarithamic plots of both the half-life and peak emission appear quite linear at high dioxane concentrations. Indeed, the total light yield, $E_0 \times t_{1/2}$, is constant to ± 3%.

The peak emission results in pure water are difficult to interpret. First, there is an apparent initial decrease in the peak emission intensity with increasing dioxane concentration. Second, there is an apparent discrepancy with the data in Figure 1, which indicates an order of magnitude increase in $E_0 \times t_{1/2}$ at 19% dioxance compared to the value in pure water. Since it is known that the emission spectra are functions of the time and concentrations, (ref. 3), it is possible that the initial decrease in the peak intensity shown in Figure 3, is associated with an initial red shift of the spectrum. Comparison of the data in Figures 1 and 3 is also made difficult by the different response curves of the IP 21 and IP 28 photomultipliers. Finally, the NaOH concentration change also affects the total emission (ref. 4).

The emission spectrum for the 16.7 vol-% solution is given in Figure 4. This emission curve was corrected for photomultiplier response by calibration against a luminol reference standard (ref. 34) in the 450-500 mμ region, and by using the IP28 response curve (ref. 6) from 500 to 610 mμ. Each data point represents a fresh sample at the emission peak for the wavelength measured, at about 5 seconds from the beginning of the reaction. However, the peak emission intensity at different wavelengths is not entirely constant as a function of time. The spectrum is generally similar to that reported by Ryzhikov (ref. 3) and Kariakin (ref. 7), but has not been compared with that from aqueous solution under our reaction conditions.

Preliminary data have been obtained for the total emission from a lucigenin flow system containing 24 vol-% dioxane. The emission intensity vs time is shown in Figure 5. The initial half-life (from the linear extrapolation to zero time emission) is about three seconds. This is reasonable agreement with the batch-mixed half-life value of four seconds.

3. Tetrahydrofuran (THF) Concentration Effects

The dependence of the peak emission, decay half-life, and "figure of merit" upon the volume per cent THF in the mixed

Table 2

SOLVENT OXIDATION*

No.	Reactant	Vol-%	H_2O_2 M/liter	NaOH M/liter	Detector	Peak Photocurrent amp	Remarks
1	MeOH	29.2	1.5	0.58	1P28	1.2×10^{-10}	
2	i-PrOH	16.7	1.2	1.00	1P28	7×10^{-10}	Spectrograde (compare No. 3)
3	i-PrOH	27.3	1.6	0.55	1P28	3×10^{-6}	Solvent grade
4	Dioxane	33.3	1.5	0.50	1P28	$>10^{-8}$	
5	Acetone	33.3	1.2	0.67	1P28	8.7×10^{-9}	Two-phase
6	Pyridine	50.0	1.2	0.33	1P28	3×10^{-9}	Two-phase
7	THF	21.6	1.2	0.50	1P21	2×10^{-6}	Visible flash
8	Acetone plus satured p-terphenyl	33.3	1.2	0.67	1P21	8×10^{-6}	Visible flash Two-phase
9	Acetone plus saturated tetraphenyl-butadiene	33.3	1.2	0.67	1P21	10^{-6}	Visible flash Two-phase
10	None	-	1.2	0.67	1P21	5×10^{-10}	
11	Acetone	33.3	1.2	0.67	1P21	8.1×10^{-9}	
12	Luminol†				1P28	7.75×10^{-5}	

* All reagents are "high purity" grades except as marked (No. 3)

† Standard Luminol reference solution concentrations in mole/l: luminol, 4×10^{-3}; $K_2S_2O_8$, 6×10^{-2}; H_2O , 3×10^{-2}; Na CO , 0.1.

†† Vol-% organic in aqueous solution.

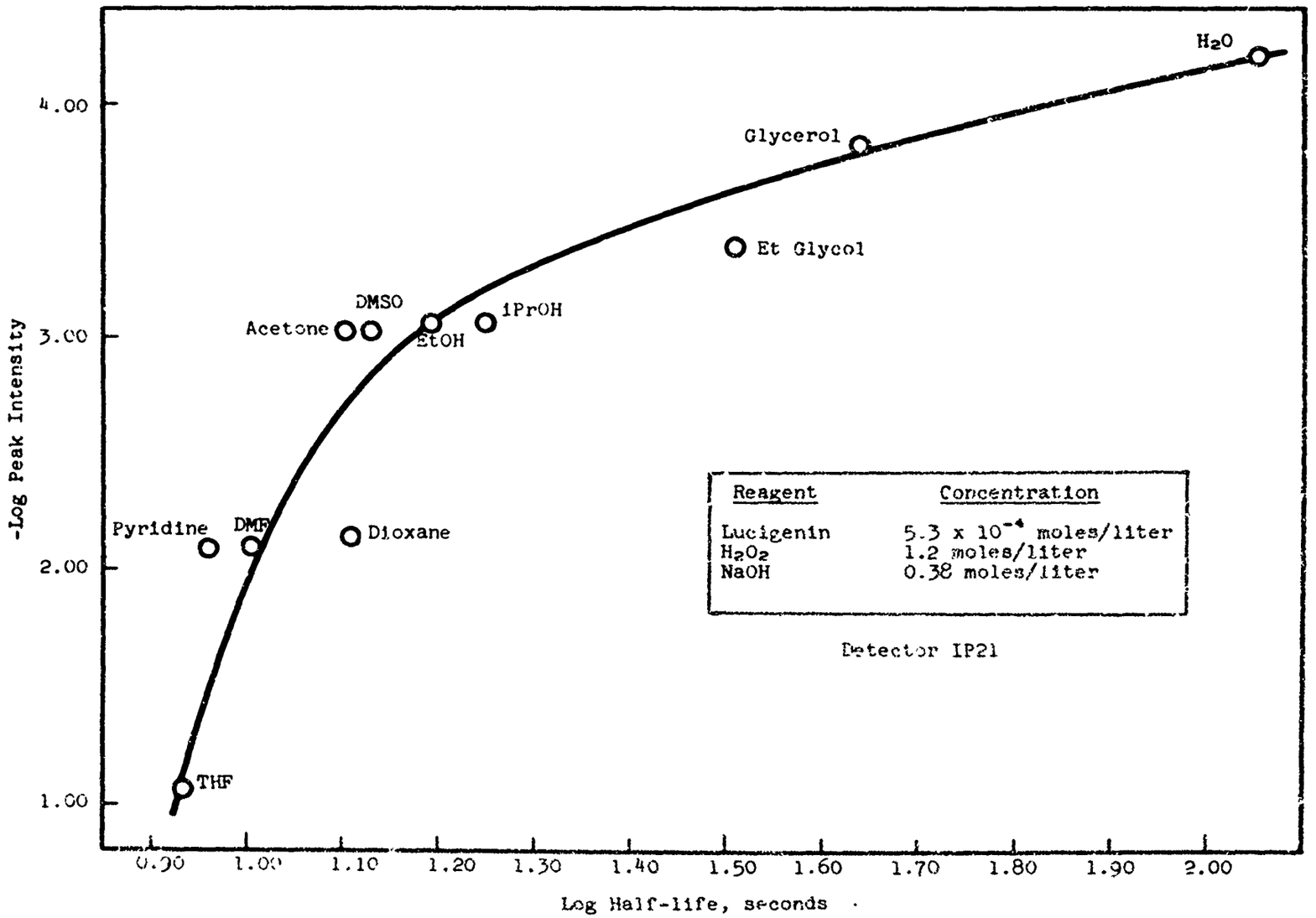


Figure 2. Peak Intensity for Lucigenin Oxidation in Mixed Solvent (18.7 vol-% organic) vs Half-life for Emission Decay

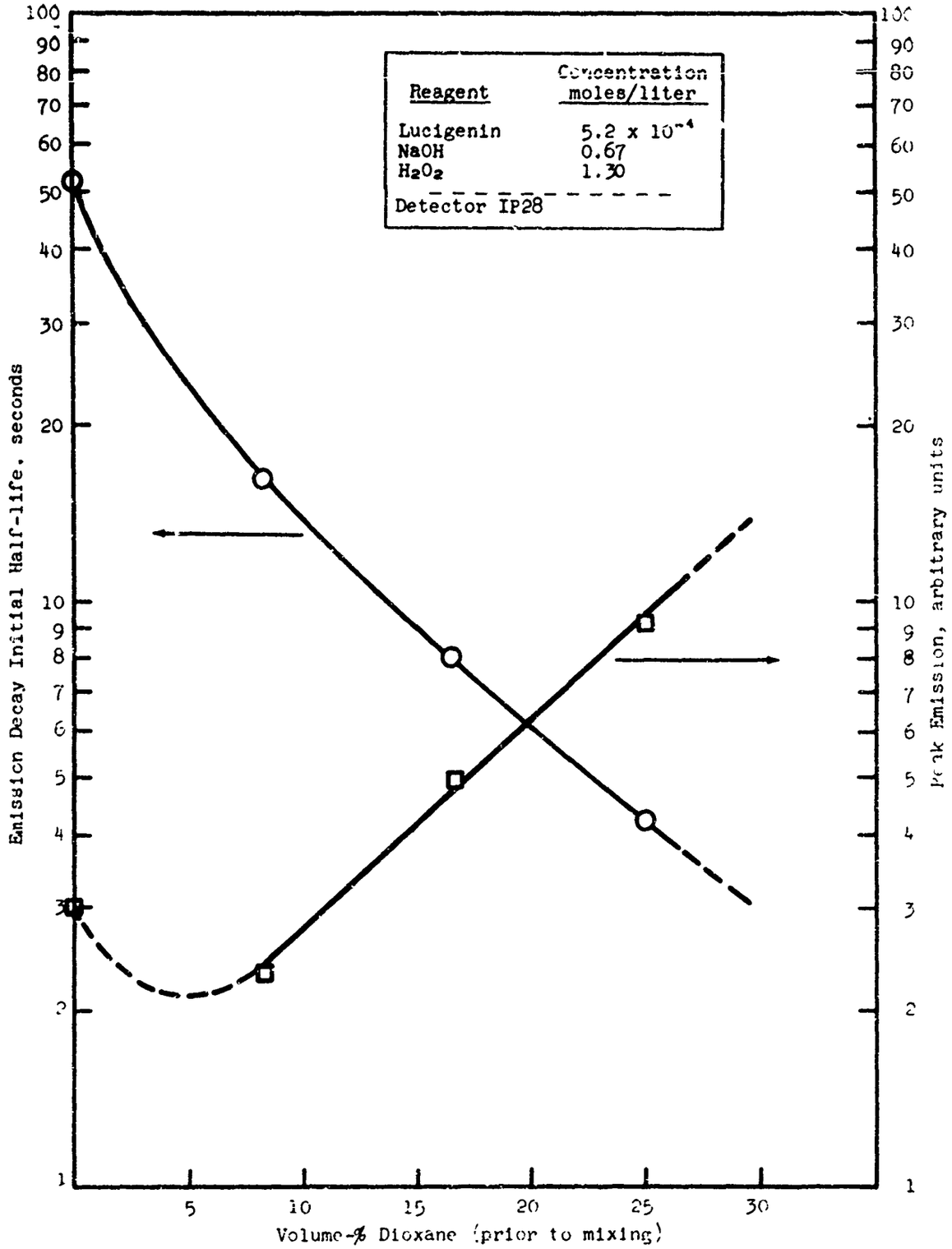


Figure 3. Peak Emission and Half-life for the Dioxane "Catalyzed" Lucigenin Oxidation

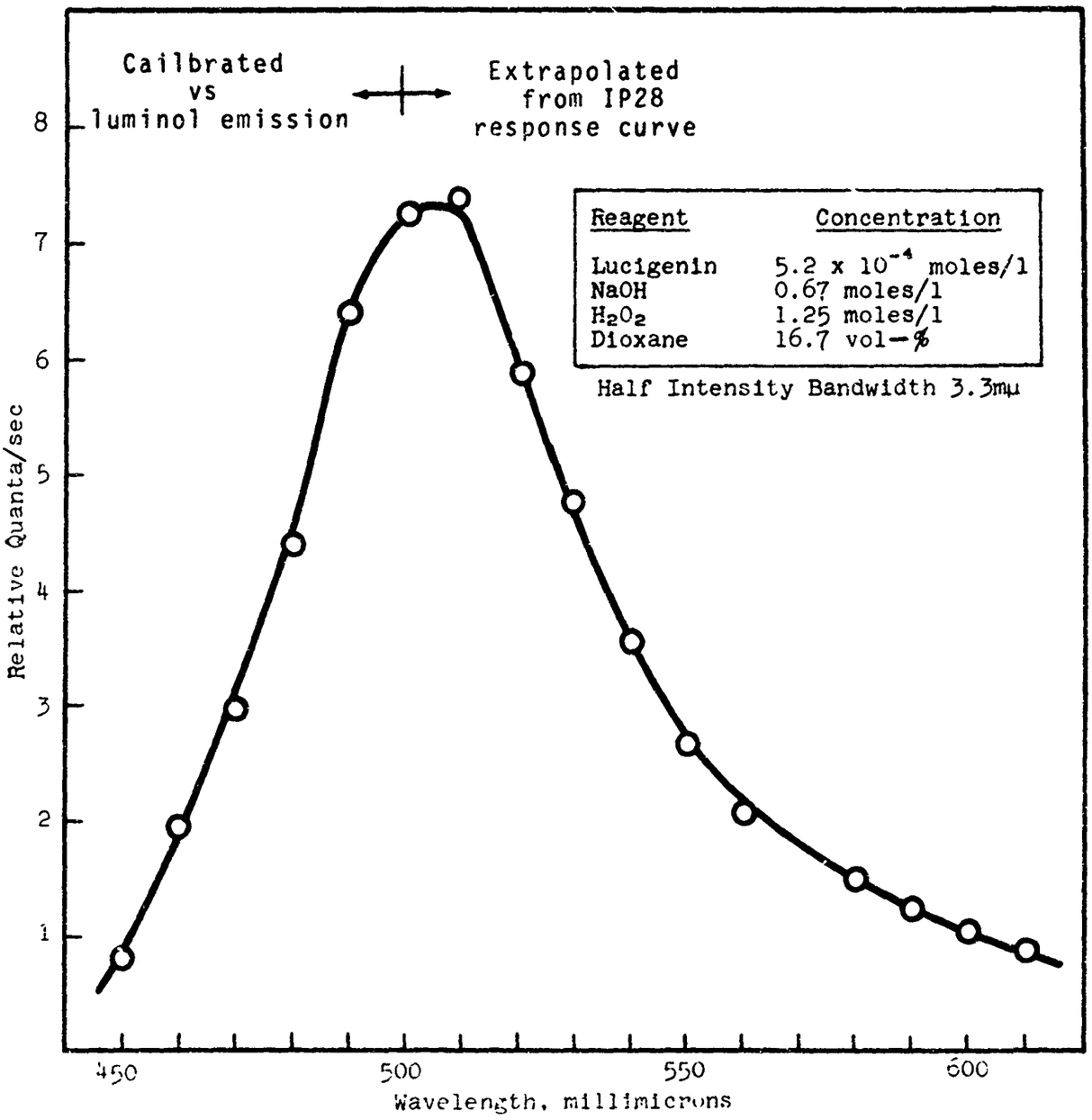


Figure 4. Lucigenin Peak Emission Curve
(Corrected for system response)

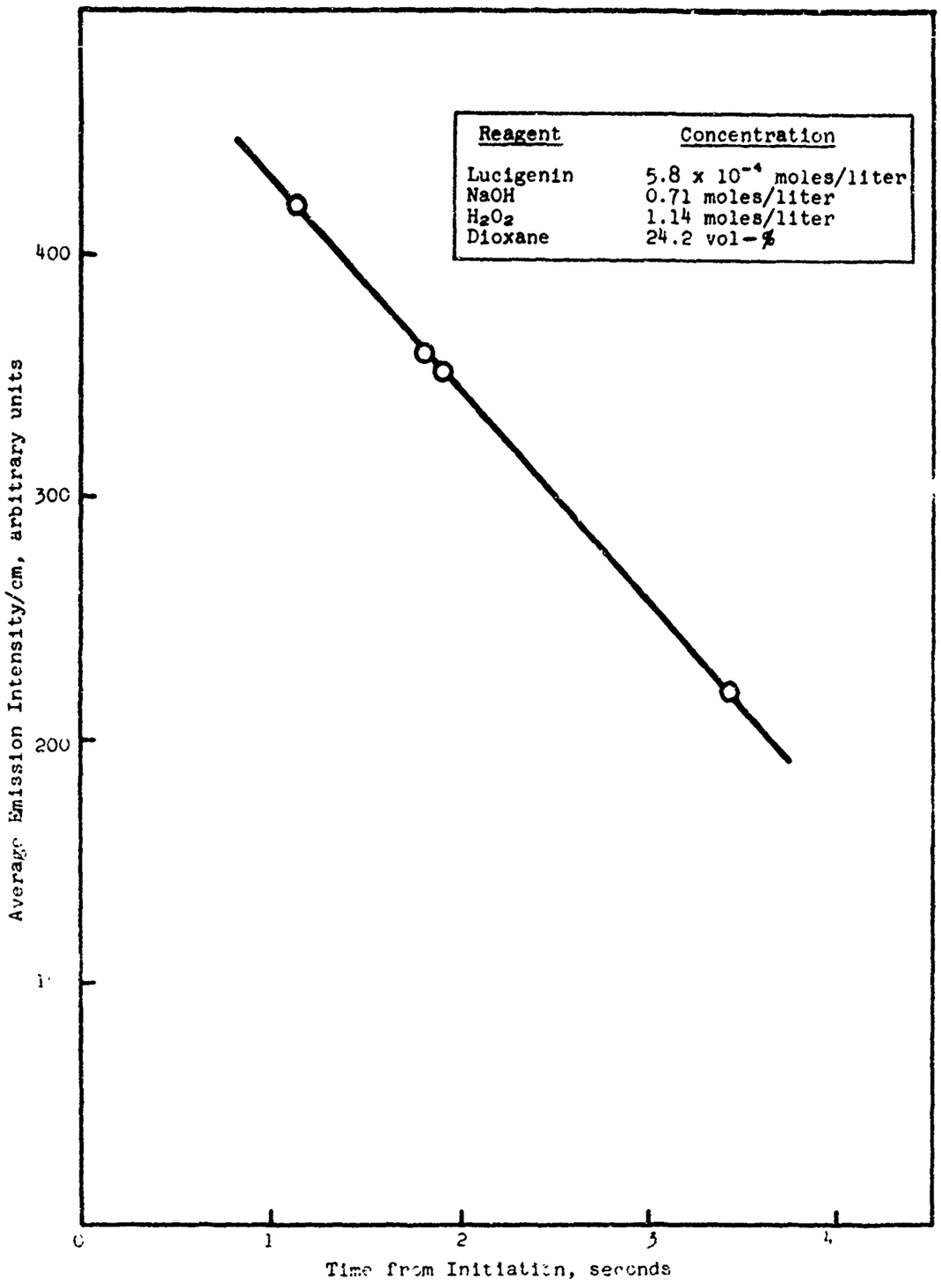


Figure 5. Gross Emission vs Time for Lucigenin Oxidation in Flow Reactor

solvent is presented in Figures 6-8. Both emission peak intensity and decay half-life are monotonic functions of the THF concentration. The figure of merit which is related to the integrated output, is a simple, increasing function of the THF concentration only within the region defined by absence of reaction product precipitation.

Within the limitations of the simple "figure-of-merit" product (rather than the integrated emission), the efficiency of the emission appears to increase by a factor of about two until reduced by turbidity, assuming that the emission spectrum is unchanged. The logarithmic dependence of the decay half-life upon the THF concentration over a rather wide range is striking. For a pseudo first-order reaction (ref. 4), this relationship is formally that due to a decrease in activation energy. This may, of course, be determined by measurement of the reaction rate temperature coefficient at various THF concentrations. It would clearly be of interest to investigate this apparent efficiency increase for additional solvents in which greater reaction product solubility may exist.

In the course of determination of the background emission from alkaline peroxide solvent solutions, it was observed that the decay half-life of the presumably lucigenin-free aqueous solutions was strikingly similar to that of the lucigenin-containing solutions (Table 3).

Table 3

DECAY HALF-LIVES OF ALKALINE PEROXIDE SOLUTIONS

(H_2O_2 conc. = 1.2 mole/liter)

Reagent Level		Decay Half-Life, sec.*	
NaOH, M	THF, vol %	Lucigenin, 4×10^{-3}M	Lucigenin-free
0.38	0	115	90
0.67	0	38	38
0.67	13	8.0	8.2
0.67	24	3.8	3.1

* Note that the peak intensity ratios for the decays compared above are greater than 10^4.

It is not clear at this point whether this striking correlation is a trivial result of contamination of lucigenin-free solutions. If the observations were reproducible under various conditions,

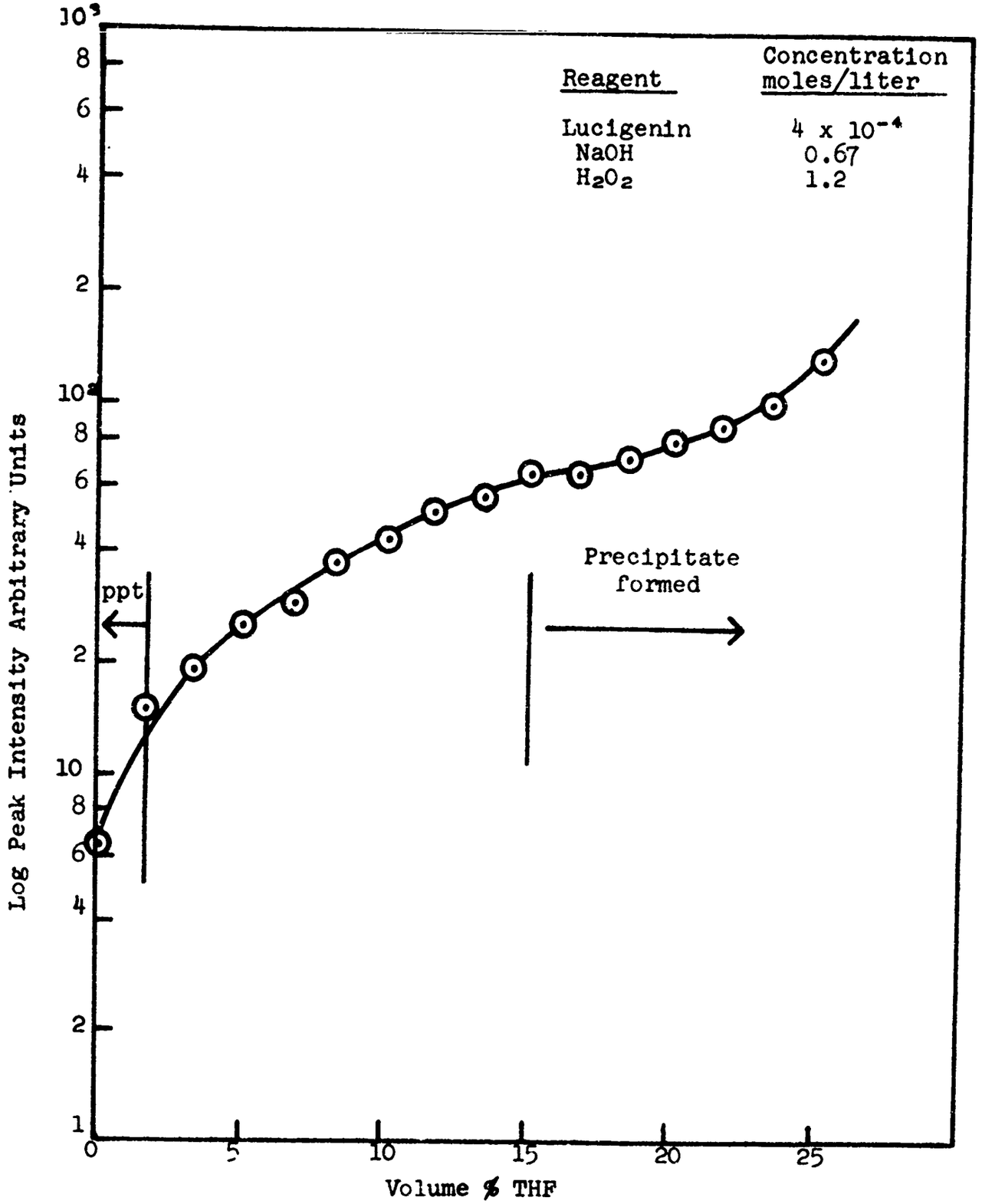


Figure 6. Peak Emission of Lucigenin as a Function of THF Concentration

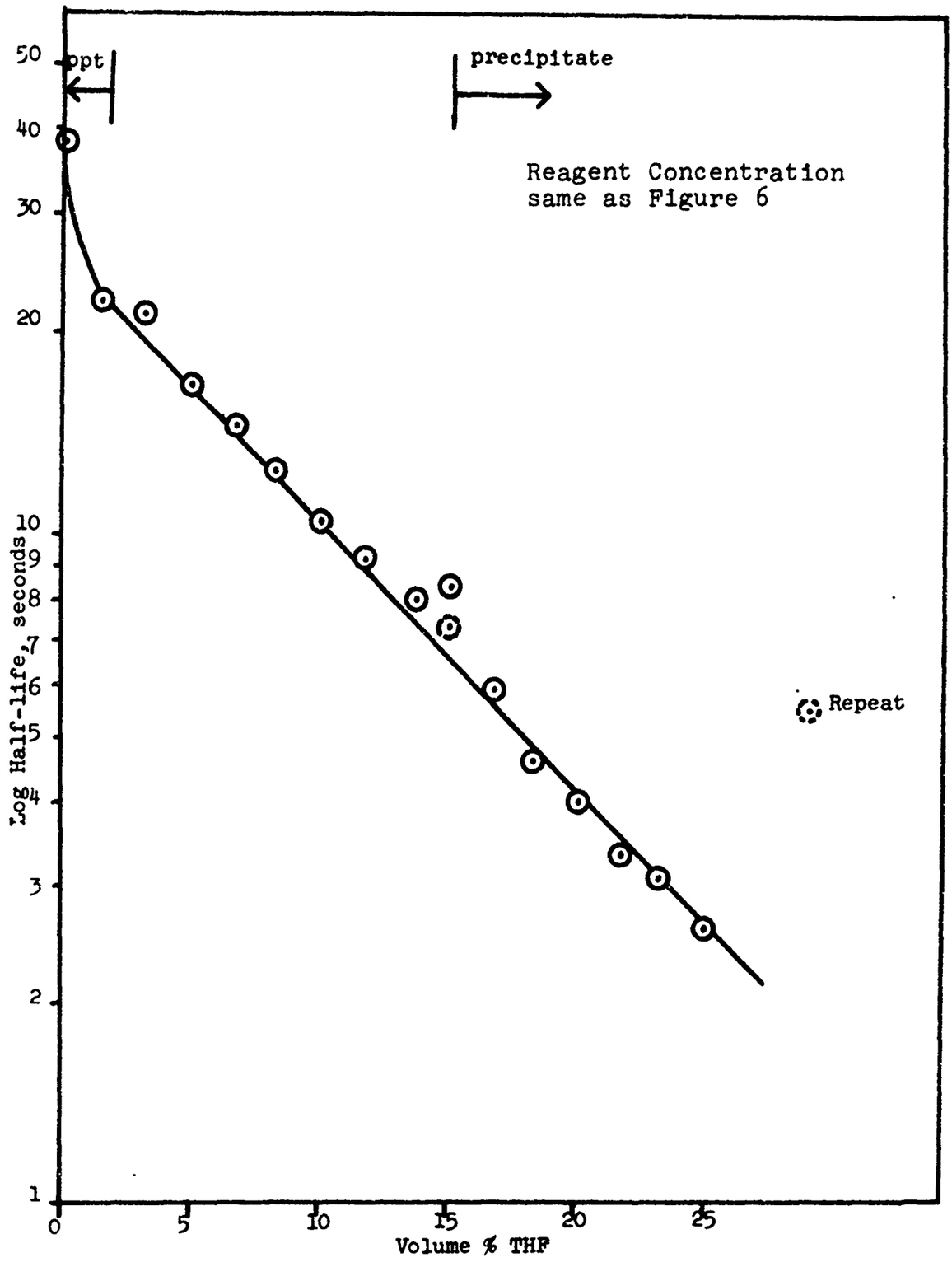


Figure 7. Half-life of Lucigenin Emission as Function of THF Concentration

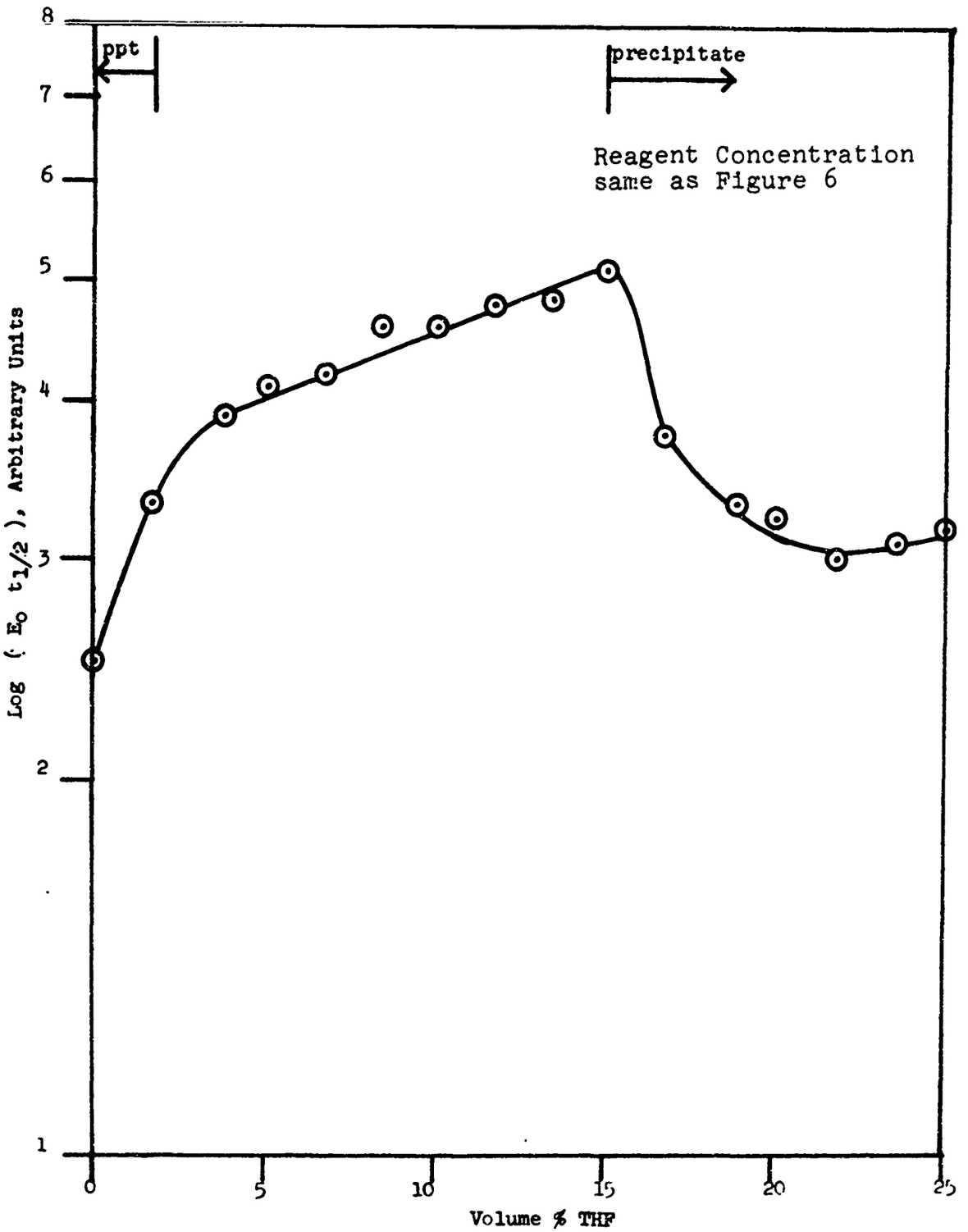


Figure 8 Variation of the E_o ($t_{1/2}$) "figure of merit" of Lucigenin with THF Concentration

it would strongly suggest that the luminescence of lucigenin is a sensitization, or energy transfer phenomenon.

4. Nonaqueous Solutions of Lucigenin

A comparison was made between total emission from air-saturated lucigenin solutions in absolute methanol in the presence of alkali alcoholates and aqueous alkaline solutions. The results are shown in Table 4.

Table 4

LUMINESCENCE OF AIR-SATURATED LUCIGENIN SOLUTIONS
(1.3×10^{-3} M/l)

Solvent	Base	Base Molarity	Peak Photocurrent (amp)
MeOH	NaOMe	0.33	2.6×10^{-7}
MeOH	t-BuOK	0.10	3.1×10^{-7}
H_2O	NaOH	0.67	6.7×10^{-8}

No major changes in the peak photocurrent were noted.

E. ALKALINE PEROXIDE OXIDATIONS OF LOPHINE

Preliminary studies were performed on the peroxide-hypochlorite oxidation of lophine in mixed alcohol-acetone-water solvent. Great difficulty was encountered in obtaining reproducibility, and the study was discontinued. Although part of the reproducibility problem was later traced to instrumental instabilities, it was observed that relatively rapid decomposition of lophine occurred in a variety of solvents. The "half-life" of a 10^{-4}M solution of lophine in dimethylsulfoxide (DMSO) or dimethylformamide (DMF) appeared to be about one hour, as determined by ultra-violet spectrophotometry.

The following qualitative observations are included for completeness.

The dependence of the peak emission (E_0) and emission decay in half-life ($t_{1/2}$) upon hydroxide ion concentration is shown in Figure 9. The product $E_0 \times t_{1/2}$, which may be taken as a crude figure of merit for total output, decreases monotonically from the lowest hydroxide ion concentration examined.

The gross emission spectrum (not corrected for photomultiplier response) is plotted in Figure 10 with commerical bleach as the

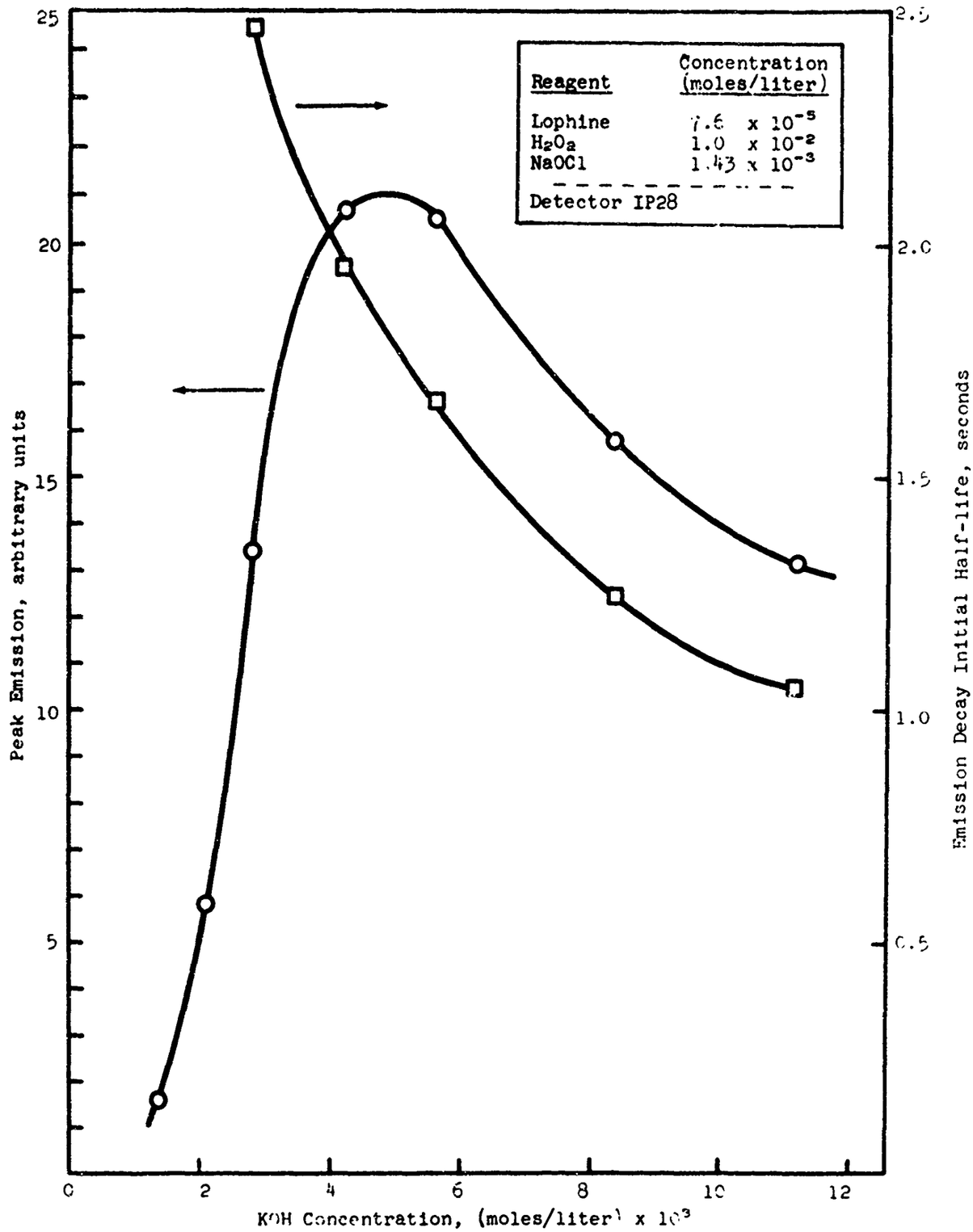
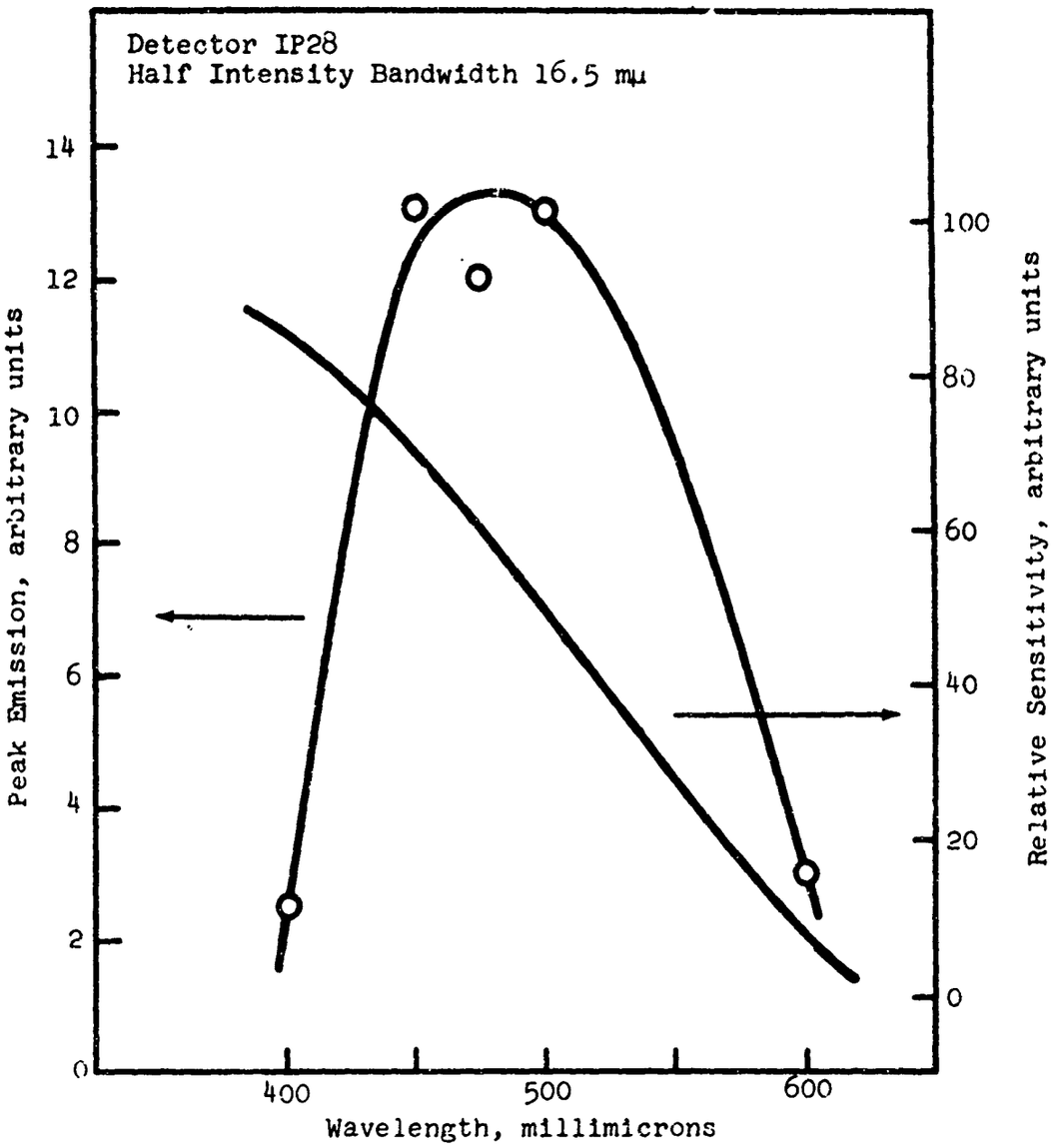


Figure 9. Peak Emission Intensity and Emission Decay Half-life as a Function of (KOH) for Lophine Oxidation



Reagent	Concentration, moles/liter
Lophine	9.6×10^{-4}
H_2O_2	1.0×10^{-2}
NaOCl	6.2×10^{-2}
KOH	1.15×10^{-2}

Figure 10. Approximate Peak Emission Spectrum of Lophine (uncorrected)

hypochlorite ion source. The values given are for the peak response at the given wavelength.

Chemiluminescence from lophine in DMSO and DMF was observed for t-butyl peroxide oxidation in the presence of t-butyl alcoholate. The luminescence was much weaker than in the aqueous alcohol-acetone solvent. However, the concentrations employed were probably far from optimum.

F. ALKALINE PEROXIDE OXIDATIONS OF PYROGALLOL

The alkaline peroxide oxidation of pyrogallol produced a weak luminescence. Pyridine and heavy metal salts are found to increase the emission intensity. The results are given in Table 5. The light intensities were found to be at best three orders of magnitude lower than that produced from luminol.

Table 5

PYROGALLOL OXIDATION*

No.	Reactant	Catalyst	Remarks	Peak Photocurrent, amp
1	Pyrogallol 3×10^{-3}M	$FeSO_4$	Phosphate buffer, pH 6	2.4×10^{-10}
2	Pyrogallol 3×10^{-3}M	Pyridine, 1.6×10^{-2}M	Acetate buffer, pH 6.5	2.4×10^{-9}
3	Pyridine 1.6×10^{-2}M	None	-	7.2×10^{-11}
4	Pyrogallol 3×10^{-3}M	Pyridine 8×10^{-3} CrAA 2×10^{-4}**	Acetate buffer	7.1×10^{-9}***
5	Pyrogallol 3×10^{-3}M	Pyridine 8×10^{-3} Cu Phthalocyanine	Acetate buffer	7.1×10^{-8}

*H_2O_2=0.3M for all reactions; detector 1P28

**AA is the acetylacetonate complex

***Similar results with FeAA. CoAA peak photocurrent 2.5×10^{-9} amp

III. CHEMILUMINESCENCE SCREENING PROGRAM

A. INTRODUCTION

A number of organic compounds are known to chemilumenescence under the proper conditions. The structures of these materials are quite diverse. It is presently impossible to predict whether a given compound will have a chemiluminescence emission great enough to be of interest in practical field applications. However, a certain amount of direction toward the selection of potentially useful materials can be obtained from studying the structures of known chemiluminescent compounds and their reaction conditions. It was on this basis that we began a screening program in a search for new, bright chemiluminescent systems.

A general criterion for visible chemiluminescent reactions is that the transient metastable species possess energy at least 40 kcal per mole greater than that of the products in order to satisfy the minimum energy required for emission in the red region of the spectrum. If for any reason the excited molecules cannot radiate, addition of molecules capable of receiving this energy (fluorescers) that are themselves efficient fluorescence emitters is necessary.

During the course of a chemiluminescent reaction, several species may be formed. The structural complexity of the fragments will determine the type and amount of excess energy each receives from the bond-breaking and bond-forming processes. If a molecular fragment has many functional groups that are interbound in a manner such that many resonance structures can be written, the probability exists of many intersecting potential energy surfaces. This would facilitate the ease of thermalization of excess energy through intersystem crossing. Conversely, a small molecule will have fewer paths for thermalization, and the radiation probability will increase. Small molecules that might be eliminated are CO_2, CO, N_2, O_2, and H_2CO. The emission spectra of these molecules are well known. It is quite possible that these molecules do not take up excess energy since their lowest excited electronic energy levels may be greater than the energy available in the bond-forming and breaking process. In this case, the organic species may accept a larger share of the energy as opposed to the situation where its dissociation partner is also a large molecule. The organic species should be a simple fluorescent molecule. The luminol system illustrates the elimination of a small molecule (N_2).

The wavelength emitted and fluorescence efficiency are other factors to be considered in selecting candidate compounds. While oxidation systems producing simple, excited molecules (e.g., CO_2) can sometimes be made bright if the proper sensitizer is found, the majority of known chemiluminescent reactions involve the production of more complex aromatic organic species with

fluorescence capabilities. Logical choices in a screening program, therefore, should emphasize molecules which might be expected to be oxidized to products with high fluorescence efficiencies.

Based on the above guidelines, several classes of compounds appeared to be promising. Derivatives of fluorene, the acyloins, heterocyclics (such as benzoxazoles and indoles), and polarized aromatic olefins were logical starting points in the screening program.

B. GENERAL OBSERVATIONS

1. Reaction Medium

The solvent-base system in which an autoxidation reaction is carried out greatly influences the emission of a chemiluminescent reaction. Much of the previous work in chemiluminescence was done in aqueous solution. We chose dimethyl sulfoxide (DMSO) rather than water as our main reaction medium for several reasons. Preliminary experiments showed that an enhanced light output was possible in DMSO (see Section II). In addition, DMSO is a better solvent for a majority of the organic compounds proposed for study.

Dimethylformamide (DMF) was also tested as a reaction solvent. The emission from this system was often the equal of that from DMSO, but DMF was more prone to oxidative decomposition than was DMSO.

Potassium t-butoxide (t-BuOK) proved to be the best base for effective chemiluminescence emission in DMSO solution.

2. Solvent (Background) Chemiluminescence

No chemiluminescence emission is observed when O_2 is dissolved in pure DMSO. However, the addition of potassium t-butoxide (t-BuOK) produces a pulse at 7×10^{-11} amp†, which drops quickly (one minute) to a steady level of $\approx 4 \times 10^{-11}$ amp. Addition of more O_2 leads to a slow rise to 8×10^{-11} amp (at a flow rate of $\approx$ 0.35 cc/sec) in about 15 minutes. This background value must be kept in mind in discussing subsequent reactions.

The baseline chemiluminescence of DMF is an order of magnitude greater than that of DMSO. This is also true of t-BuOK solutions of DMF-DMSO mixtures. It is clear from the instability of these solutions that extensive decomposition of DMF occurs. Comparison of the emission from fluorene and 9-fluorenone in DMF and DMSO, however, reveals enhanced emission in DMF, again emphasizing the importance of solvent variation in optimization.

† Data for batch photometer with P28 photomultiplier at 500 volts.

C. EXPERIMENTAL RESULTS

1. General

Chemiluminescence from the autoxidation of organic compounds appears to be the usual, rather than an exceptional, occurrence in aprotic media. Almost all the compounds screened gave emissions greater than that of the background (solvent) reaction.

Generally, the emission was observed in two components. Upon addition of the final reagent (base), an "initial flash" with a half-life of 2-3 seconds was observed. Continued addition of oxygen resulted in a build-up of the "true" chemiluminescent emission which normally had a lower peak intensity and a much longer half-life than the initial flash. The cause of the initial flash is unknown, but it could be connected with the presence of a small amount of DMSO peroxides.

Results of the screening program are given in Appendix IV. Data for approximately 175 compounds are given there. The screening results of a number of indole derivatives, not included in the Appendix, can be found in the section concerned with the detailed study of indole chemiluminescence (Section IV).

As previously stated, the general conclusion of the screening program is that almost every compound tested showed at least weak chemiluminescence in the range covered by our IP28 detector. The great majority of compounds screened had chemiluminescent intensities too weak to be developed into a practical system. There were, however, in addition to the indoles, two classes of compounds which showed relatively bright emissions. These were acyloin derivatives and electronegatively substituted olefins.

2. Acyloin Chemiluminescence

a. Structural Effects

The luminescence observed for the substituted acyloins supports the view that a new class of chemiluminescent reactions has been discovered, Table 6 and 7.

Table 6 Chemiluminescence of Selected Acyloins

Compounds	Reaction Conditions†	I/Io
Benzoin		0.10
Benzoin	t-BuOK = 0.016	0.20
Benzoin		4×10^{-2}
Benzoin	in DMF	0.15
Benzoin	DPA	0.10
Benzoin	DPA in DMF	0.20
Benzoin	fritted gas dispersor	0.15

(continued on next page)

Table 6 Chemiluminescence of Selected Acyloins (Continued)

Compounds	Reaction Conditions†	I/Io
Anisoin		$(6.6\pm0.8)\times10^{-2}$
Anisoin		0.15
4,4'Dihydroxybenzoin	DPA	2.4×10^{-2}
4,4'Dihydroxybenzoin	TP or DPA	4×10^{-2}
2,2'Dihydroxybenzoin	DPA	10^{-2}

† 5×10^{-3}M Benzoin, 0.1M t-butoxide in DMSO
DPA = 9,10-diphenylanthracene
TP = p-terphenyl

Anoisoin appears to be similar in emission intensity to benzoin, although quantitative comparisons are difficult to make in the absence of quantitative spectra. Furoin, because of less stabilization of the radical formed, is clearly a weaker emitter. A very striking effect is noted for 4,4'-dihydroxybenzoin. The intense absorption noted for the other compounds are presumed to be that characteristic of the free radical is absent. Nevertheless, both the emission intensity and induction period are of the same order as observed for benzoin and the closely related anisoin. 2,2'-Dihydroxybenzoin, which does form the dark solution, has an (unsensitized) emission intensity lower by almost an order of magnitude from that of the 4,4'-isomer. Although more detailed investigation of these effects is required to understand the phenomena, one may speculate that oxidative side reactions producing quinones may drastically reduce the emitting species. The advantageous decrease in self-absorption by the clear solution may thus be cancelled out by a sharp fall in the reaction rate of the chemiluminescent reaction.

The observed sensitivity of the peak luminescence intensity to the procedure details such as stirring, oxygen flow rate, and degree of dispersion is probably accounted for by the competing rates of the chemiluminescent reaction (presumably the oxidation of the free radical anion) which emits radiation and the overall rate of oxidation which reduces the self absorbance.

b. Luminescence Spectra

Typical luminescence spectra of benzoin, benzil, and oxidized benzoin in DMSO are given in Figures 11 and 12. Major emission peaks in these uncorrected spectra are seen at 370, 425 and 505 $\pm$ 10 mμ.

The salient feature of these spectra is the coincidence between the chemiluminescence emission at 505 mμ and the benzil fluorescence emission at the same wavelength. Backstrom (ref 45)

Table 7

CHEMILUMINESCENCE OF ACYLOINS

Reaction Conditions:

5×10^{-3}M in DMSO
t-BuOK 0.1M
O_2 flow rate ≈0.3 cm^3/sec

sample volume 30 cm^3
cross section 2.5 cm
fritted gas dispersor

Compound	Initial Pulse 100 I/I_o	Time to Peak*	Peak Intensity, 100 I/I_o	$t_{1/2}$*	Remarks
4-Dimethylaminobenzoin	0.2	23 s	40.0	8 s	Same color sequence as benzoin
Benzoin	0.1	14 s	15	8 s	
Anisoin	0.1	22 s	6.5	7 s	
4,4'-Dihydroxybenzoin	0.24	30 s	2.4	60 s	colorless solution
2,2'-Dihydroxybenzoin	1.8	18 s	0.45	18 s	
		3 m	0.22	2.8m	second peak
Furoin	0.8	5 m	0.3	7 s	capillary bubbler
Acetoin	0.24	45 s	0.12	-	
		5 m	0.13	5 m	second peak
4,4'-Dihydroxy-3,3'-dimethoxybenzoin	6.0	5 s	0.34	5 m	colorless solution
3,3'-Dichloro-4,4'-dihydroxybenzoin	3.0	5 s	0.50	25 s	colorless solution
Blank	0.2	20 m	0.02	long	

* s = seconds, m = minutes

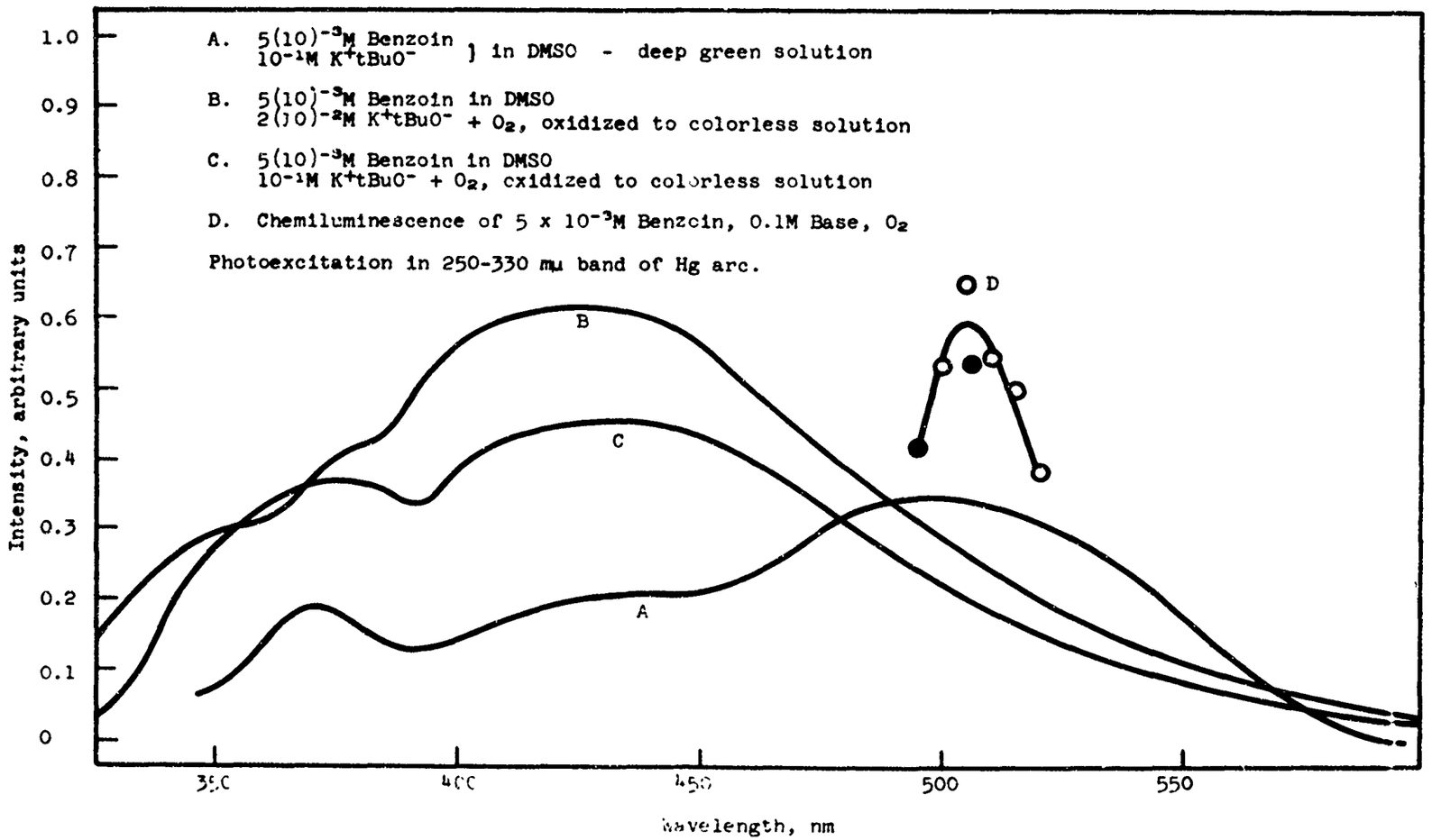


Figure 11. Photoluminescence Spectra of Oxidized Solutions of Benzoin; Chemilumunescence Spectrum of Benzoin Autoxidation

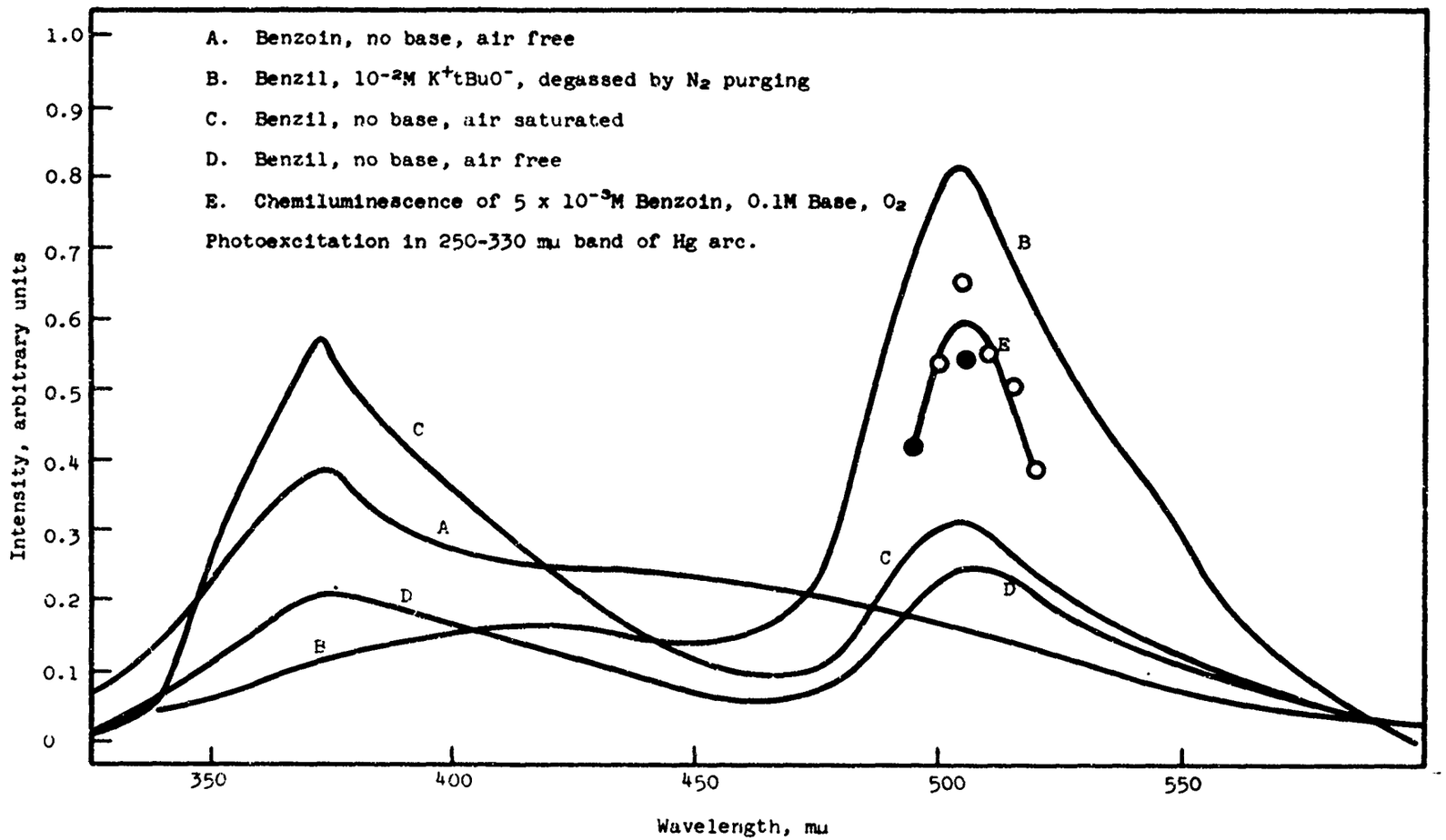


Figure 12. Photoluminescence Spectra of 5 x 10^{-3}M Benzil, 5 x 10^{-3} Benzoin and Chemiluminescence Spectrum of Benzoin Autoxidation

has analzyed the luminescence spectra of a number of diketones and has shown that the fluorescence emission of benzil in benzene peaks at 508 mμ. Our measurements of the absorption spectrum of benzil in base-free DMSO confirm the absence of a major solvent shift and lead, therefore, to the assignment of the 505 mμ peak to the singlet transition or fluorescence spectrum of benzil.

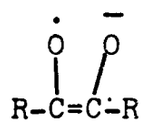
With the exception of this identified fluorescence, the spectra are at present not amenable to analysis, since the intensities and peaks observed apparently reflect a rather complex chemistry, and are found to be dependent upon reaction variables such as hydroxylic species (OH^-, t-BuOH, and H_2O) and time. Thus, the 370 mμ peak generally observed in both benzil and benzoin is absent in Figure 12. Deliberate addition of water (1%) has been found to accentuate this peak.

The absence of the 505 mμ benzil fluorescence peak in the oxidized ebnzoin samples indicates that either a relatively rapid rearrangement occurs, or that benzil may be only a minor product of the chemiluminescent reaction.

The chemiluminescence spectrum observed appears to rule out, a major contribution from phosphorescence, reported by Backstrom to the peak at 565 mμ in oxygen-free benzene.

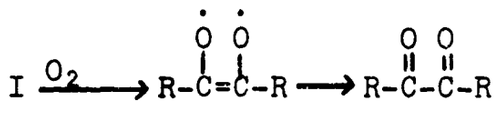
c. Oxidation Mechanism

The acyloins have been subject to considerable study (ref. 38,39). The resonance-stabilized aromatic radical anion (I) has been identified as an intermediate.



(I)

by ESR spectra (ref. 40,41). This work has recently been extended to the alicyclic (ref. 42) and alkyl (ref. 43) derivatives. Chemiluminescence appears to be associated with the oxidation of the radical anoin. This assumption is supported by the qualitative observation that the luminescence intensity peak occurs with the discharge of the characteristic intense radical-anoin absorption. It therefore becomes plausible that at least some fraction of the excitation energy required for chemiluminescence is derived by the direct oxidative excitation mechanism proposed by Chandross (ref. 44). This process may be written as:



(II) (III)

where the biradical (II) is an excited state of (III).

The chemiluminescence spectrum, on the simplest assumption, should therefore be identical with the luminescence spectrum of photoexcited benzil under the same conditions.

3. Vinyl Compounds

Vinyl compounds containing electronegative substituents have been found to undergo autoxidation with the emission of chemiluminescence. The pertinent data are summarized in Table 8 for these compounds and some related weaker emittors.

Acrolein, $H\{CH=CHCHO)$, crotonaldehyde, $CH_3\{CH=CHCHO)$, and cinnamaldehyde, $\emptyset\{CH=CHCHO)$ yield relative emission intensities of 200, 66, 1, respectively. The effect of the substitution may perhaps be correlated with the size of the substituents. Substitution of groups adjacent to the olefinic linkage in methyl vinyl ketone produces similar trends. The phenyl derivative, in this case, diminishes the intensity to a tenth of that observed for the parent compound. Replacement of the vinyl group with methyl (giving the compound acetone) essentially eliminates chemiluminescence.

The carboxylate anion does not appear to be effective in promoting chemiluminescence; for example, there is no significant difference in the luminescence of the saturated and unsaturated dibasic compounds e.g. succinic, and maleic or fumaric acids.

Table 8

CHEMILUMINESCENCE OF VINYL COMPOUNDS

Conditions:

0.1M K^+tBuO^- in DMSO + O_2
conc. reactant - 5×10^{-3}M

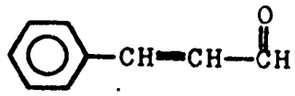
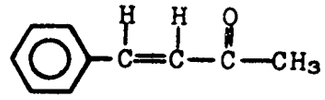
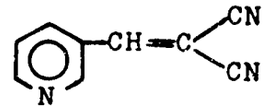
Compound	Structure	Initial Pulse 100 I/Io	Peak Intensity, 100 I/Io	$t_{1/2}$†	Remarks
Acrolein (stabilized with hydroquinone at 100 ppm)	$H_2C{=}CH{-}\overset{O}{\overset{\|}{C}}H$	8.0	7.0	11 s	lt red after reaction
Crotonaldehyde	$H_3C{-}CH{=}CH{-}\overset{O}{\overset{\|}{C}}H$	2.6	2.7	6 s	dk red after reaction
Cinnamaldehyde		15.0	4×10^{-2}	—	lt red after reaction
Methyl vinyl ketone	$H_2C{=}CH{-}\overset{O}{\overset{\|}{C}}{-}CH_3$	2.4	8.2	21 s	dk red after reaction

Table 8 (Continued)

Compound	Structure	Initial Pulse 100 I/Io	Peak Intensity, 100 I/Io	$t_{1/2}$ +	Remarks
Trans-4-phenyl-3-buten-2-one		0.8	0.8	17 s	lt red after reaction
Acrylonitrile	$CH_2{=}CH{-}CN$	0.1	1.2	75 s	dk red after reaction
Crotonitrile	$CH_3{-}CH{=}CH{-}CN$	2.6	2.7	45 s	dk red after reaction
3-Pyridylmethylene-malonitrile		0.64	3.6	4 m	--

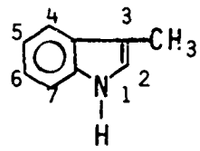
+ s = seconds
m = minutes

IV. THE CHEMILUMINESCENCE OF INDOLE DERIVATIVES

A. INTRODUCTION

The screening program described in the preceding section was designed to facilitate the discovery of new chemiluminescent systems. The indoles, the acyloins, and certain olefinic compounds were found to have emission intensities high enough to be worthy of further study. More detailed studies of these systems (described in Section III) showed the non-aqueous oxidation of indole and its derivatives to be the most promising.

Skatole (3-methylindole) (IV), had the highest total light emission



(IV)

among the readily available indole derivatives. While brightness of the skatole system is only 1% of that of luminol, skatole emission has a longer half-life so that the total light emission from skatole is about 10% that of luminol.

It was felt that a detailed study of skatole emission parameters,along with an investigation of substituent effects would provide information useful in designing a bright chemiluminescent system.

B. THE CHEMILUMINESCENCE OF SKATOLE SOLUTIONS

1. General Considerations

The indole oxidation results obtained by Totter (refs. 5,8) in both aqueous and dimethylsulfoxide(DMSO) solutions suggested an evaluation of the autoxidation reaction in potassium t-butoxide (t-BuOK) - DMSO solution. The two media are compared in Table 9.

Table 9

SKATOLE LUMINESCENCE IN AQUEOUS AND DIMETHYLSULFOXIDE SOLUTIONS

Solvent (Vol-% H_2O)	Base Concentration (M)	I/I_0	Remarks
11	0.55 KOH	9	Probably 2 phases
2	0.067 KOH	14	" " "
0	0.67 t-BuOK	60	

The decay half-lives were about 290 ± 10 seconds in either solution. The increase in brightness observed in the nonaqueous solvent suggested that further enhancement of the chemiluminescence might be obtained by optimizing the experimental parameters.

2. Dependence of Peak Intensity on Reactant Concentrations

The dependency of the peak emission intensity upon skatole concentration at a fixed base concentration is shown in Figure 13 while the effect of base variation (at fixed skatole concentration) on emission intensity is given in Figure 14 for two partial pressures of oxygen.

Figure 13 shows that a deviation from linearity occurs at skatole concentrations greater than about 2×10^{-4}M (i.e., at 3:1::t-BuOK:skatole).

A more exact figure is derived from the base variation experiments (Figure 14). The maximum brightness is obtained at a base to skatole mole ratio of one. At higher mole ratios (10-20), the peak brightness falls, in part as a result of self absorption of the solution. The emission peak also shifts from 494 mμ in 0.007M base to 507 mμ in 0.1M base. The dependence of the maximum peak brightness ratio for the two oxygen concentrations is approximately proportional to the partial pressure of oxygen.

The relative light yield behavior appears to be more complex. The total emission, within the limitations of the figure of merit $[It_{1/2}/I_0]$ at optimum base concentration is about 50% greater for air than for oxygen. The reason for this is unknwon.

3. Dependence of Peak Intensity on the Nature of the Solvent

The concentration dependence of the peak brightness in 0.1M t-BuOK solution is given in Figure 15 for skatole in DMSO, dimethylformamide (DMF), and hexamethylphosphoric triamide (HPT). Data are also given for 2,3-dimethylindole and indole-3-acetic acid in DMF. The data are presented as the figure-of-merit per mole in Figure 15. This latter parameter is approximately related to the quantum efficiency of the reaction, assuming that the emission spectrum and the order of reaction are constant. This assumption is probably true in the low concentration region, but large deviations would be expected at high concentrations. The optimum heterocyclic concentration for the brighter systems is about 10^{-2}M.

The emission decay half-lives (Table 10) were not reproducible and are difficult to interpret.

The chemiluminescence of several indoles was investigated in HPT. In Table 11, the results are compared to the values observed in DMSO (see following sections). The variations found

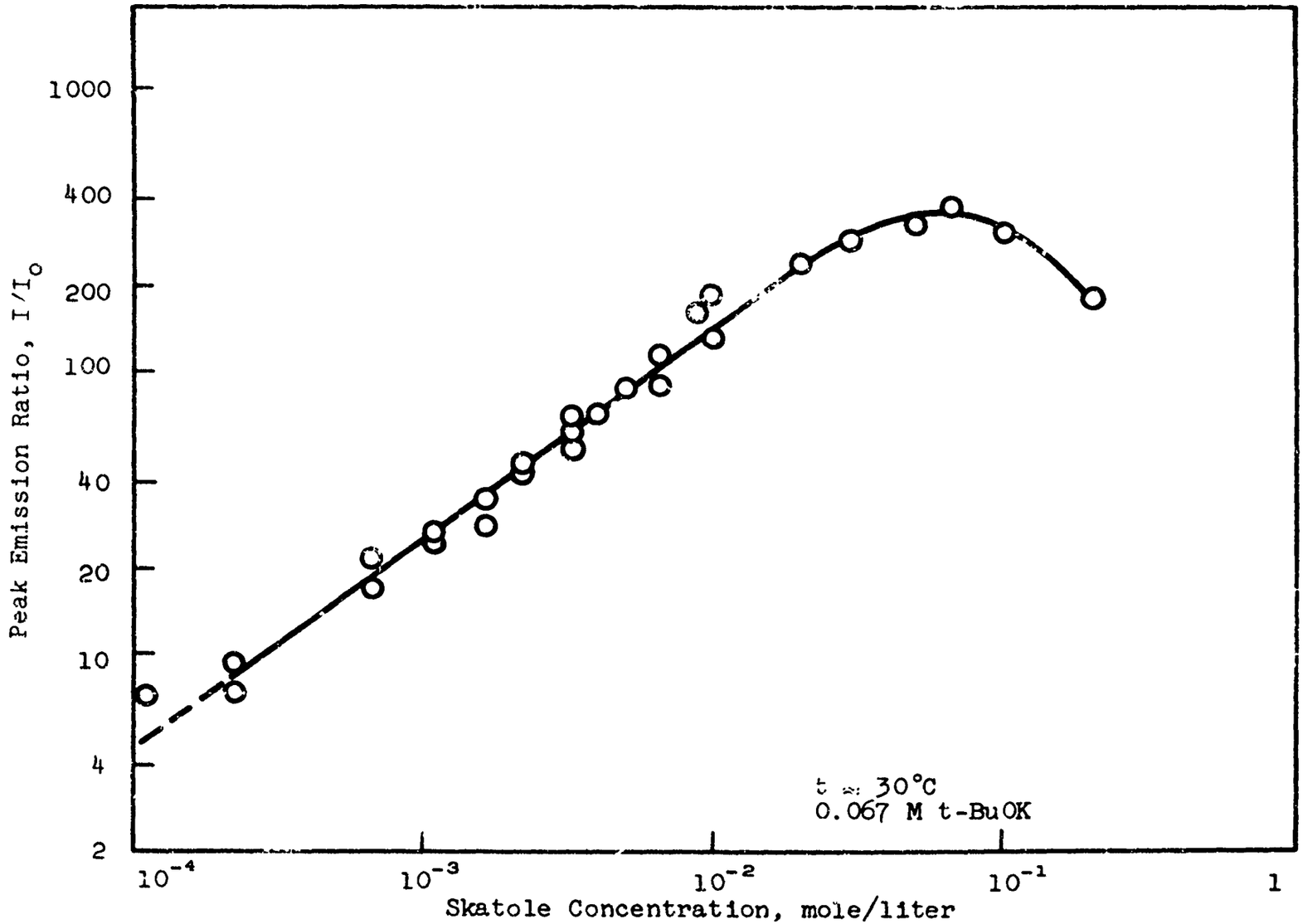


Figure 13. Peak Emission Intensity for Skatole Autoxidation in DMSO As a Function of Skatole Concentration

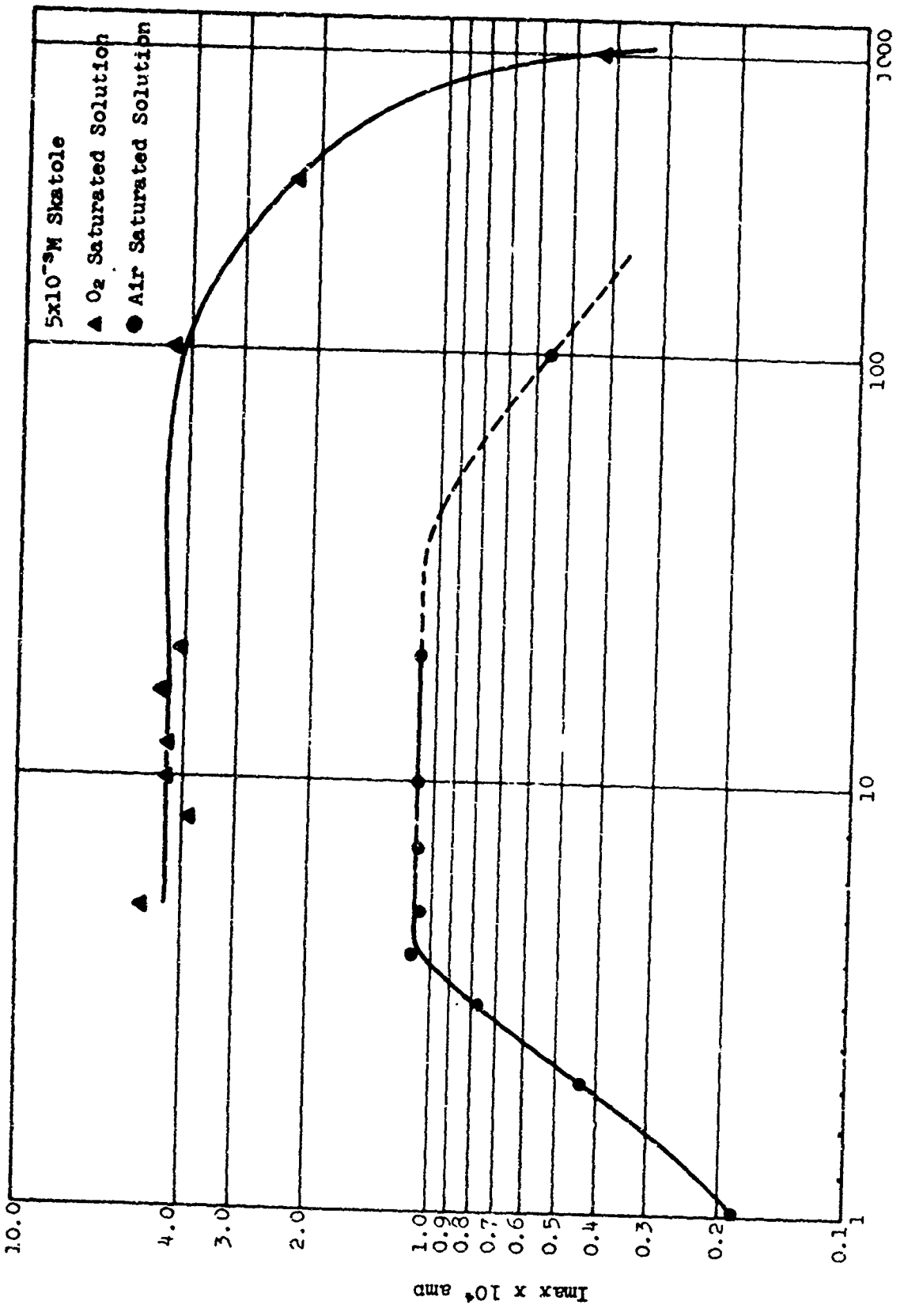


Figure 14 Dependence of Skatole Peak Brightness on Base Concentration

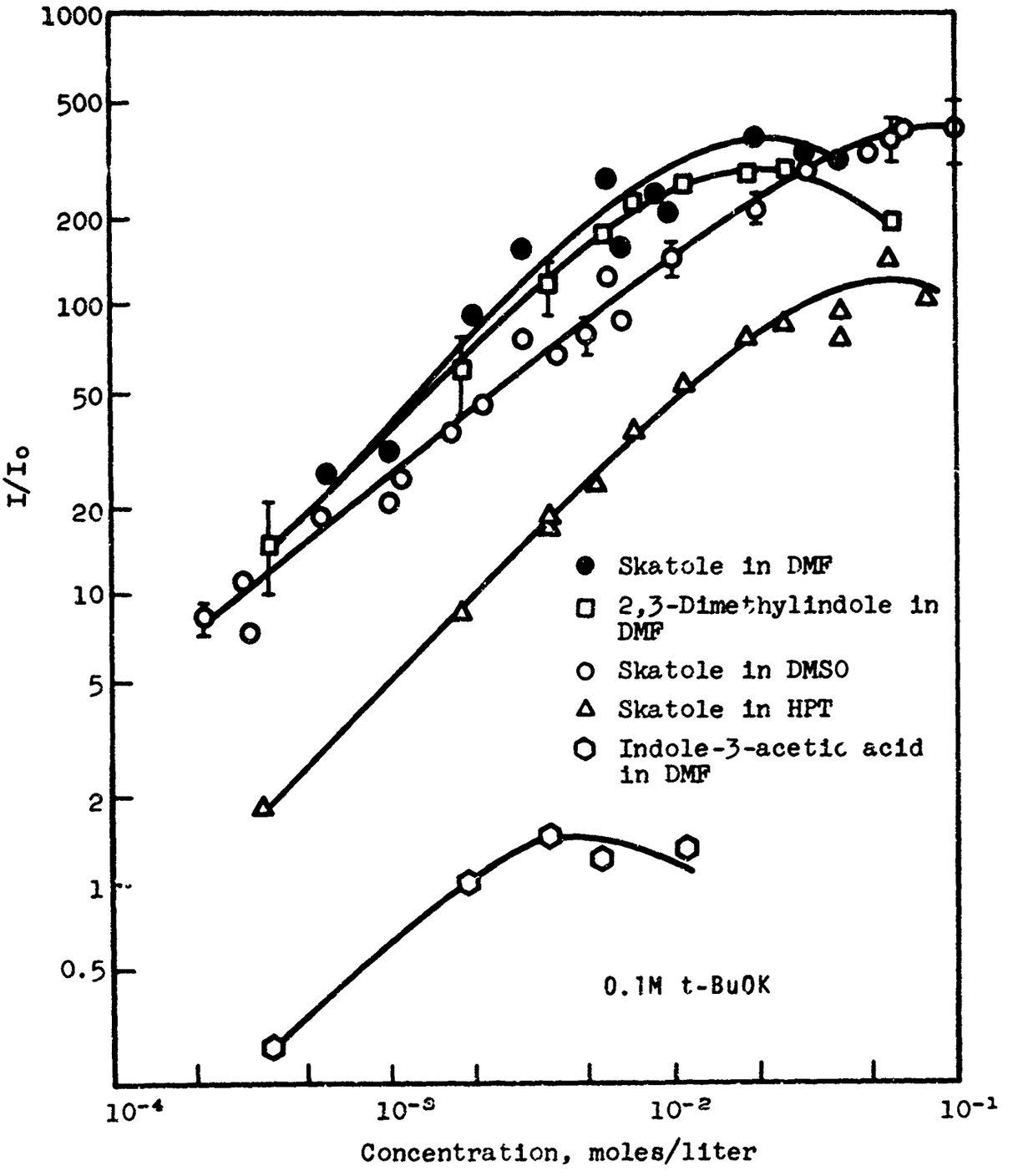


Figure 15. Concentration Dependence of Peak Brightness of Several Indoles

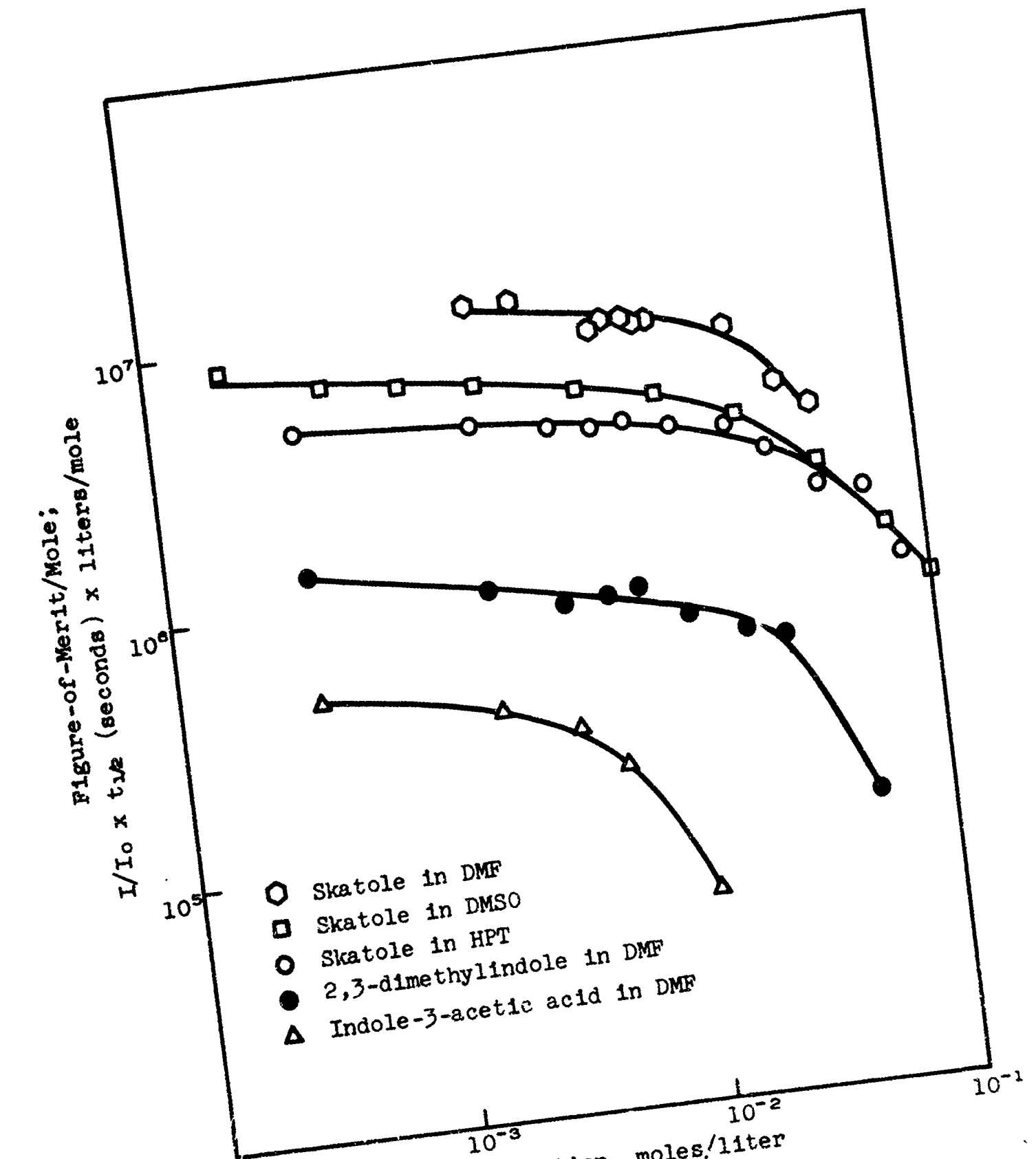
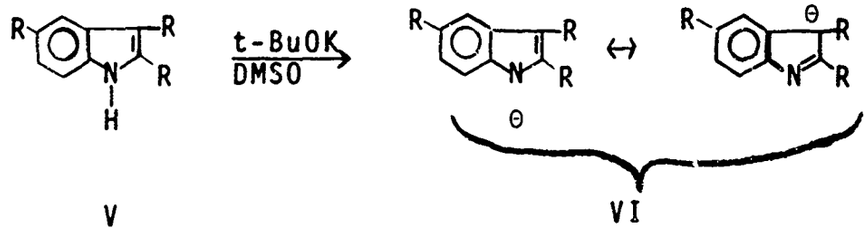


Figure 16. Concentration Dependence of Figure-of-Merit/mole for Several Indoles

C. THE IDENTIFICATION OF THE EMITTOR IN THE 3-METHYL AND 2,3-DIMETHYLINDOLE CHEMILUMINESCENT REACTION

1. Product Analyses

The fluorescence spectra of solutions of several indoles were measured as a function of chemiluminescent reaction time. The spectra for indole-5-carboxylic acid, 2,3-dimethylindole, and skatole are shown in Figures 17-19. In base-free, nitrogen-purged solutions of each indole, a short wavelength fluroescence band peaking at 350-370 mμ is observed, which corresponds to the neutral species (V). Upon addition of base, the emission peaks are shifted to 420-430 mμ. The absence of any 360-370 mμ peak in the basic solution suggests that the indole derivative is completely converted to its anionic form (VI).



V VI

Table 10

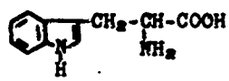
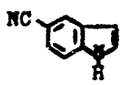
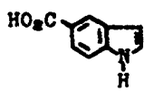
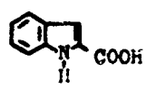
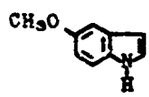
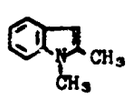
OBSERVED EMISSION DECAY INITIAL HALF-LIVES

Compound	Solvent	$t_{1/2}$ at Low Conc., (seconds) ≈5 x 10^{-4}M	$t_{1/2}$ at High Conc., (seconds) ≈7 x 10^{-2}M	$t_{1/2}$ Maximum Observed, (seconds)
Skatole	DMSO	240	200	235
Skatole	HPT	925	765	-
Skatole	DMF	240 (10^{-3}M)	450	-
2,3-Dimethylindole	DMF	30	35	40
Indole-3-acetic acid	DMF	640	540 (at 10^{-2} M)	900

Table II

CHEMILUMINESCENCE PARAMETERS OF SOME INDOLES IN HPT SOLVENT

Indole Concentration: 5×10^{-3}M
Base Concentration: 0.1M

Compound and Structure	Peak O_2 Current I/I_o	Time to O_2 Peak, seconds	$t_{1/2}$ of O_2 Peak, seconds	Figure-of-Merit, seconds	Figure-of-Merit in DMSO / Figure-of-Merit in hPT
Indole*	2.2	42	60	132	0.1*
L-Tryptophan 	0.15	300	1440	216	0.5
5-Cyanoindole 	$(8.2 \pm 1.8)10^{-3}$	30 ± 30	430 ± 290	3.0 ± 1.6	$(2.8 \pm 1.5)10^{2}$
Indole-5-carboxylic Acid 	4.6×10^{-2}	48	180	8.3	2.1×10^{2}
Indole-2-carboxylic Acid 	8.4	~0	40	336	4.5×10^{-3}
5-Methoxyindole 	6.4 ± 0.2	40 ± 10	39	245 ± 34	$6.4 \pm 0.3\ 10^{-3}$
1,2-Dimethylindole 	8×10^{-4}	240	360	0.3	10

* Datum corrected for 0.1% skatole impurity; 99.7% pure by VPC analysis.

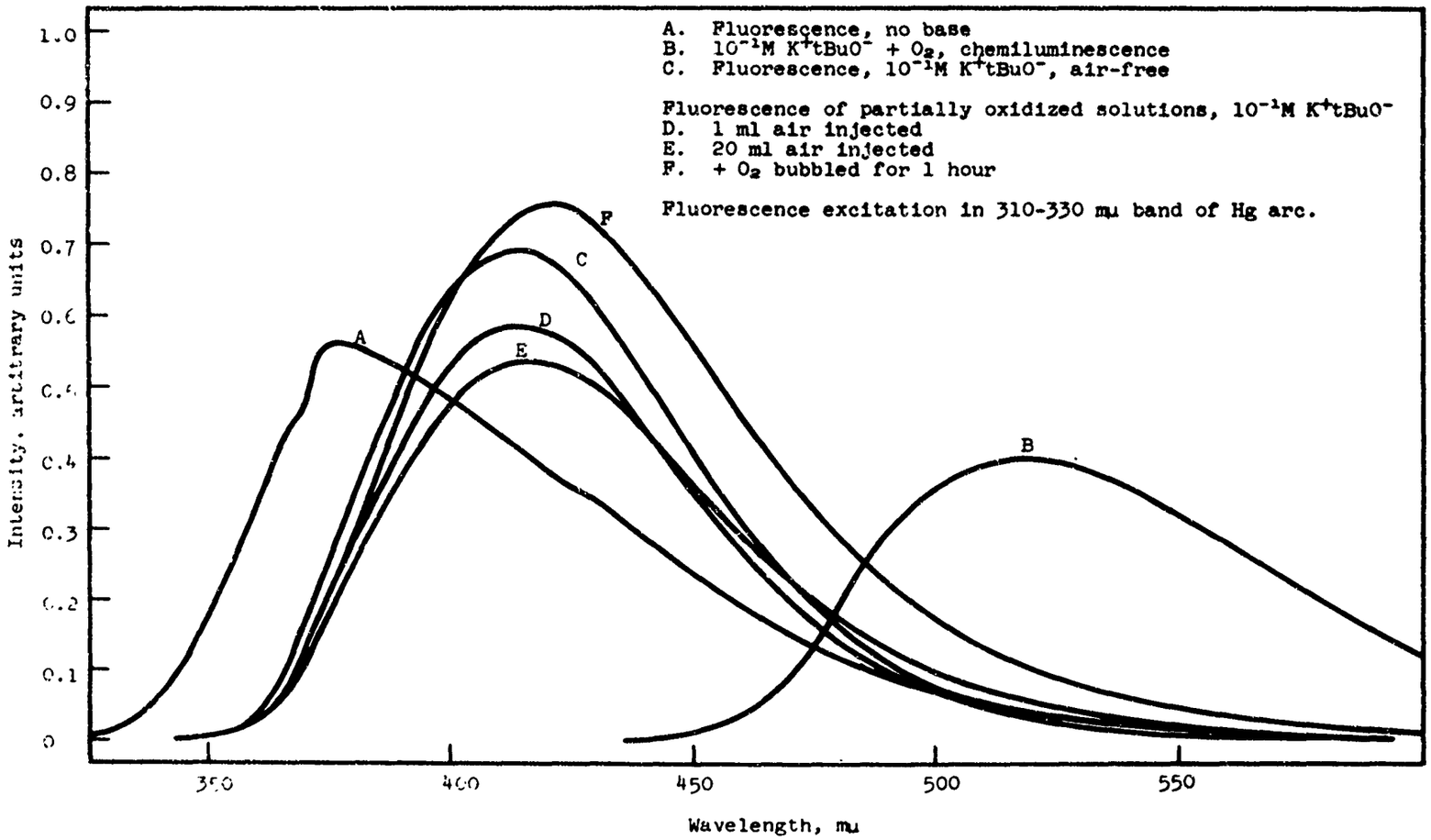


Figure 17 Fluorescence and Chemiluminescence Spectra of Indole-5-Carboxylic Acid, 5 x 10^{-5}M in DMSO

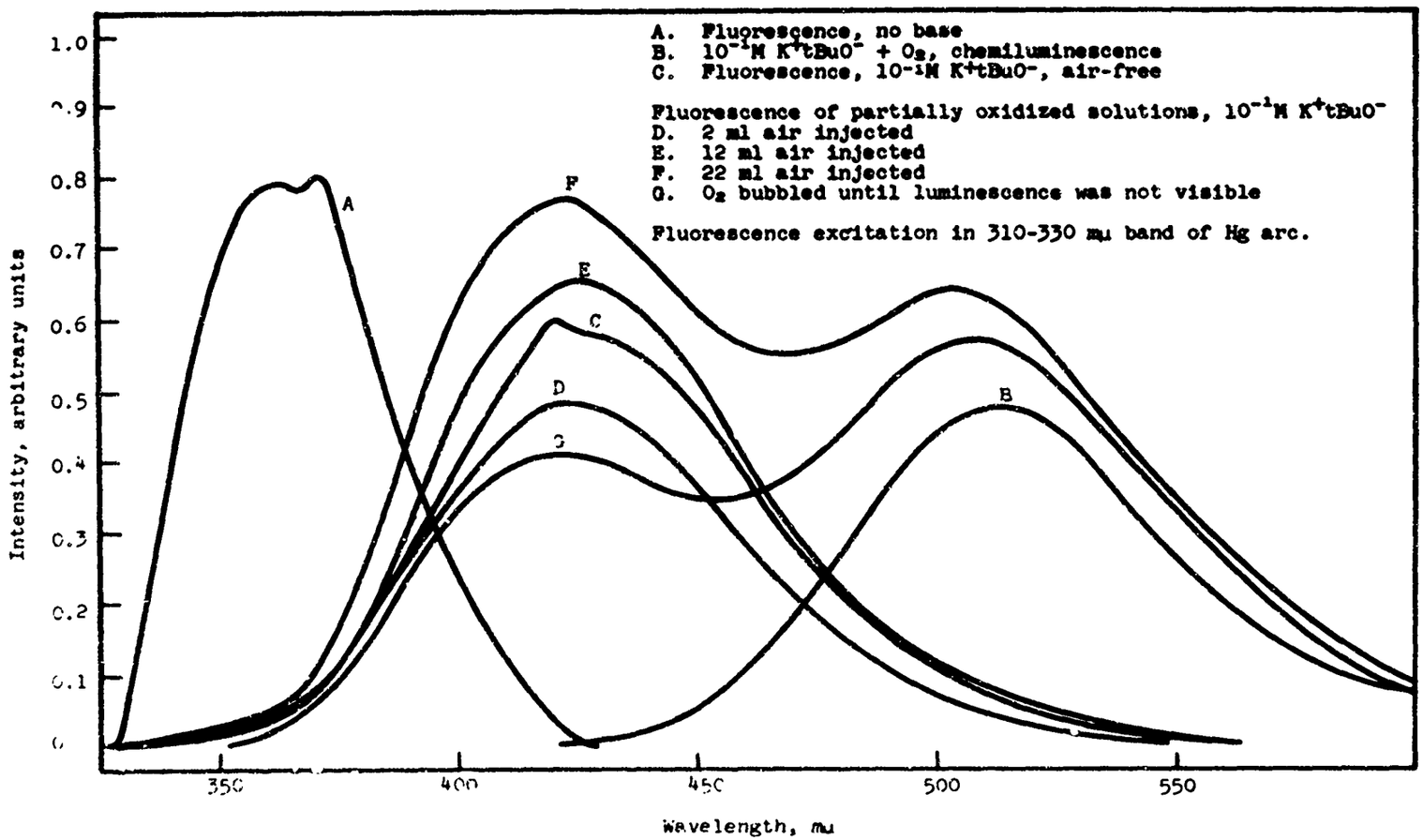


Figure 18 Fluorescence and Chemiluminescence Spectra of 2,3-Dimethylindole, 5×10^{-3}M in DMSO

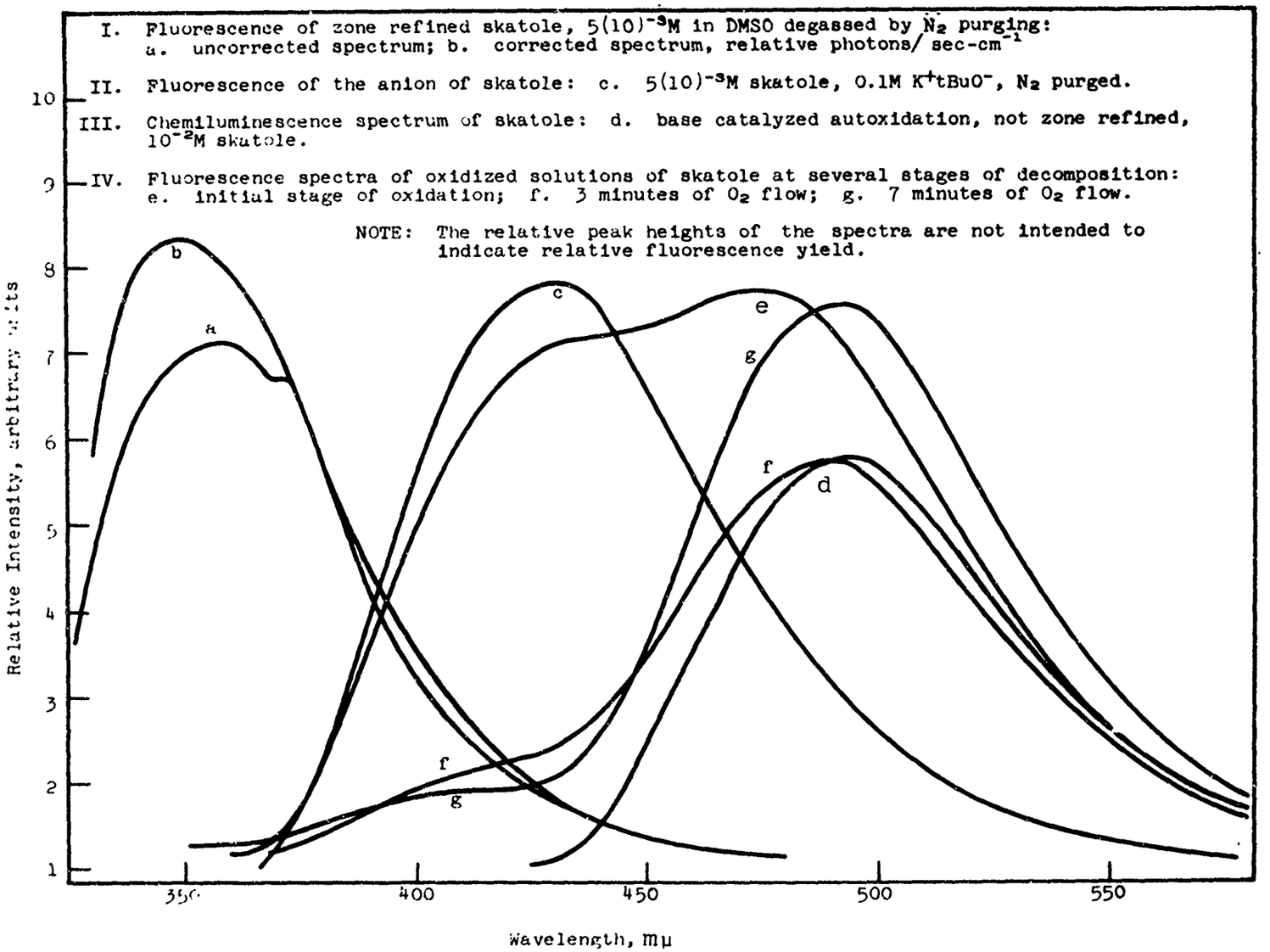
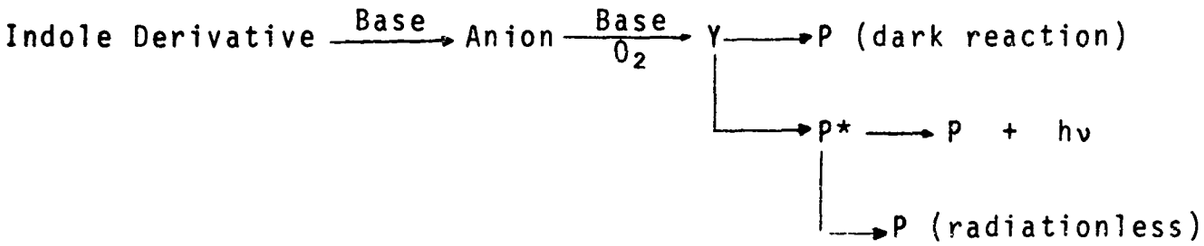


Figure 19 Chemiluminescence Spectrum of Skatole and Fluorescence Spectra of Skatole and its Oxidation Products

Small quantities of air were injected into the base-indole solution (Figure 19)*. The luminescence spectra of the partially oxidized solutions exhibit an attenuation of the anion emission. In the cases of skatole and 2,3-dimethyl indole, two new peaks were observed. For skatole, a good match is found between the chemiluminescence spectrum and the fluorescence spectrum of the oxidized solution (Curves d and g). A similar spectral agreement is found for the 2,3-dimethylindole system after the emission from the unoxidized (422 mμ band) anion is subtracted from the fluorescence spectrum of the oxidized solution (Figure 18, Curve F and G). The resultant spectrum agrees well with the chemiluminescence spectrum (Curve B).

The close similarities between the emission and fluorescence spectra suggest that the emittor is an excited state of a stable reaction product. A tabulation of additional indoles subjected to similar experiments is presented in Table 12. These data show that stable fluorescent reaction products are not formed from any of the other indoles studied. The absence of a luminescence emission band corresponding to an oxidation product may be due to the instability of the emitting molecule in basic solution, low concentration, or low fluorescence efficiency.

The overall oxidation reactions may be formulated as shown in VII where several reaction steps may be required for the production of the intermediate Y leading to the formation of product P.



VII

In no instance did the chemiluminescence emission spectrum correspond to the fluorescence spectrum of the indole derivative or its anion.

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* The quantity of air injected does not correspond to the quantity of oxygen dissolved or reacted but is related to the fraction of indole reacted.

Table 12

NCORR.CTED CHEMILUMINESCENCE AND FLUORESCENCE SPECTRA* OF INDOLES

Indole Derivative	Chemiluminescence Peak, nm	Fluorescence Peak in Neutral Solution, nm	Fluorescence Peak in Degassed Basic Solution, nm	Fluorescence Peak of Oxidized Solution, nm	Remarks
Indole	491	357 (350 in water, 330 in EtOH)[1]	394	400 394 400 393 396	2 cc of air injected into solution 7 cc of air injected into solution 12 cc of air injected into solution 17 cc of air injected into solution 22 cc of air injected into solution
5-Methylindole	523	336	407 413	406	15 minutes of O_2 flow at 0.04 cfm 60 minutes of O_2 flow at 0.04 cfm
7-Methylindole	492	374	386	384	15 minutes of O_2 flow at 0.04 cfm
3-Methylindole (skatole zone refined)	489	356 (370 in water, 350 in EtOH)[1]	430	430, 475 420, 480 420, 485	Initial stage of oxidation 3 minutes of O_2 flow 7 minutes of O_2 flow (reported in Tech Report No. 4)
3-Ethylindole	493	360	427	437	Oxygen flow for 20 minutes
L-Tryptoohan	491	370 (345,360 in H_2O)[1]	430	432	Oxygen flow for 20 minutes
2,5-Dimethylindole	492	370	406	409 424	6 minutes of O_2 flow at 0.025 cfm 25 minutes of O_2 flow
2,3-Dimethylindole in DMF	516	369 (364 shoulder) (376 in water, 360 in EtOH)[1]	423	- 514	DMF solution, 1 x 10^{-3}M indole, 0.1M base, recrystallized. Oxidation proceeded until solution was no longer chemiluminescent.

[1] B. L. Van Duuren, Chem. Rev., 63, 329 (1963).

* 313., 3[illegible]1 Å excitation wavelengths

Table 12 (Continued)

Indole Derivative	Chemiluminescence Peak, nm	Fluorescence Peak in Neutral Solution, nm	Fluorescence Peak in Degassed Basic Solution, nm	Fluorescence Peak of Oxidized Solution, nm	Remarks
2,3-Dimethylindole	514	370 (362 shoulder)	423	427, 506	Indole was oxidized until chemiluminescence emission was negligible. Data previously reported in Technical Report No. 4.
Indole-5-carboxylic acid	514	376	414	-	Technical Report No. 4
Indole-3-acetic acid	-	352 (370 shoulder	434	-	
Indole-3-acetic acid in DMF	490	353 (370 shoulder)	374	-	DMF solution.
5,6-Dibenzyloxyindole	516	-	-	-	Solution turned yellow while the fluorescence spectra were being taken.

2. Comparison of the Fluorescence Spectra of Aryl Amide Anions and the Chemiluminescence Spectra of Skatole and 2,3-Dimethylindole

The fluorescence emission spectra of several compounds that might be indole oxidation products (ref. 9) were obtained in an attempt to identify the chemiluminescent species in the 2-methyl and 2,3-dimethylindole systems. The observed emission peaks are given in Table 13.

One of the products isolated from the aqueous persulfate oxidation of indole was anthranilic acid (ref. 1). The luminescence spectrum of the neutral species (411 mμ) and of its anion (471 mμ) do not match either chemiluminescence spectrum. No correlation was found for another possible product, orthoaminoacetophenone. ψ-Indoxyl (VIII), another possible oxidation product, was not available for fluorescence measurements. However, this compound seemed unlikely as the emitter since its strong yellow-green fluorescence disappears in alkaline solutions (ref. 10).

A very good agreement was found between the chemiluminescence spectra of skatole[and 2,3-dimethylindole (IX)], and the fluorescence spectra of the anions of o-formamidoacetophenone and o-acetamidoacetophenone (X). The known reaction (ref. 32) of IX to X is shown in Figure 20.

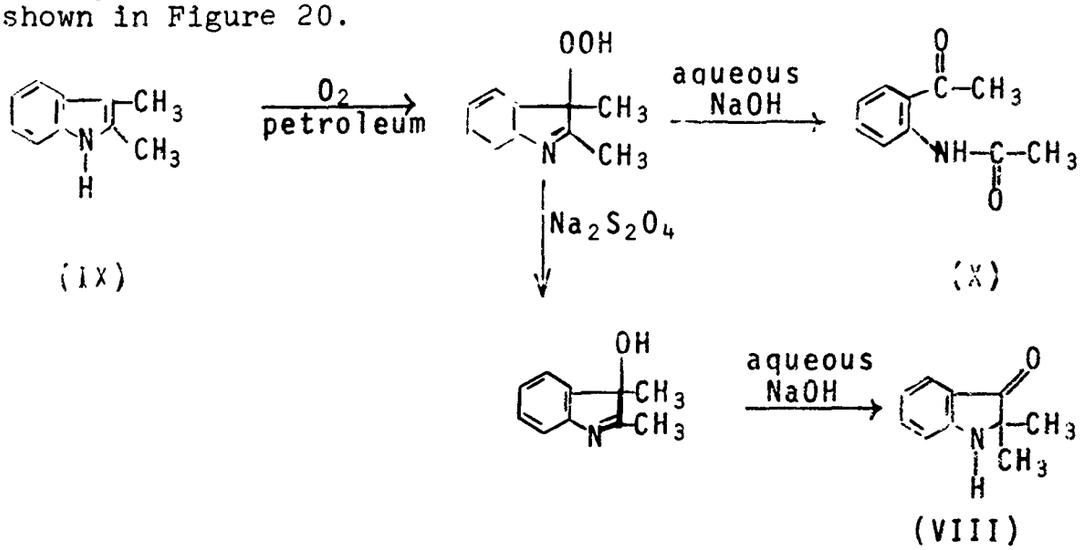


Figure 20. 2,3-Dimethylindole Oxidation Products

Confirmation of the structural assignments of the emitters in these two chemiluminescent reactions is obtained from the excellent agreement of the contours of the fluorescence and chemiluminescence spectra, seen in Figures 21 and 22. The acyamides were prepared by methods outlined in Appendix I.

The agreement between the chemiluminescence spectra and fluorescence spectra of the proposed intermediates is among the best to be found for any known chemiluminescent reaction.

Table 13

FLUORESCENCE SPECTRA OF POSSIBLE INDOLE OXIDATION PRODUCTS

Compound	Fluorescence* Peak, mμ	Remarks
Anthranilic acid	411	10^{-3} M acid, 2540 Å, 3310 Å, 3314 Å, excitation
Anion of Anthranilic acid	471	5×10^{-3} M acid, 0.1M base, air-saturated solution
Anion of N-acetyl anthranilic acid	448	5×10^{-3} M acid, 0.1M base
Anion of Oxindole	484	5×10^{-3} M oxindole, 0.1M base
Ortho aminoacetophenone	454	0.1M
Anion of ortho aminoacetophenone	454; 510 (shoulder)	0.033M compound, 0.13M base, N_2 purged solution
Oxidized 2,3-Dimethylindole	506	5×10^{-3} M, 0.1M base + O_2
Ortho acetamidoacetophenone	475; 510 (shoulder)	0.1M
Anion of ortho acetamidoacetophenone	516	0.13M base, 0.033M ortho acetamidoacetophenone
Anion of ortho formidoacetophenone	494	10^{-2} M base, 5×10^{-4} M ortho formidoacetophenone

* The 3130 and 3331 lines of low pressure mercury lamp were the exciting wavelength unless otherwise stated. Uncorrected spectra.

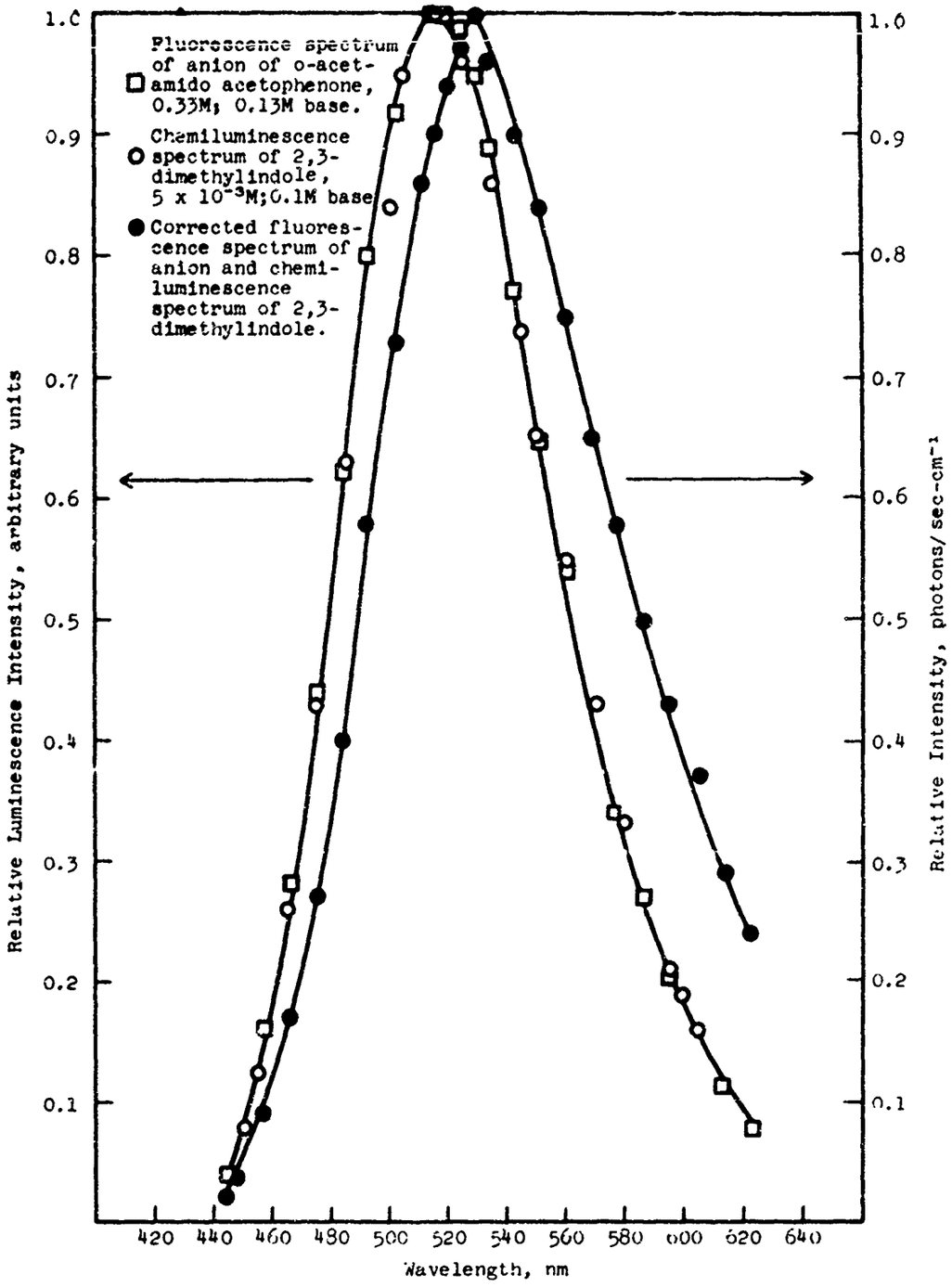


Figure 21. Comparison of the Fluorescence Spectrum of the Anion of o-Acetamido acetophenone to the Chemiluminescence Spectrum of 2,3-Dimethylindole

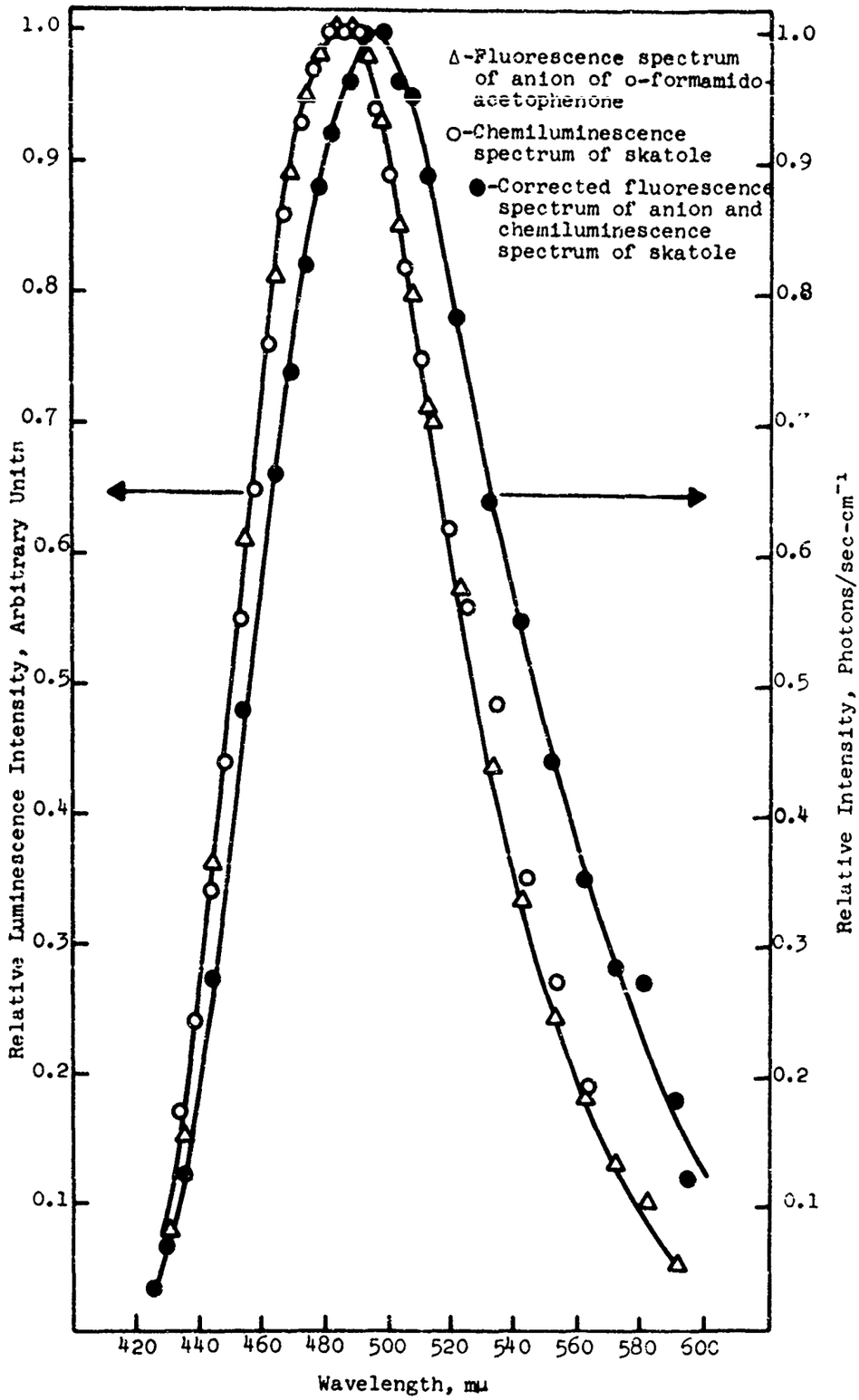


Figure 22. Comparison of the Fluorescence Spectrum of the Anion of o-formamidoacetophenone to the Chemiluminescence Spectrum of Skatole

D. THE QUANTUM YIELD OF FLUORESCENCE OF ACYLAMIDE ANIONS

The identification of the anions of o-formamidoacetophenone (FAP) and o-acetamidoacetophenone (AAP) as the emitting species in the skatole and 2,3-dimethylindole chemiluminescent reactions, prompted a study of the fluorescence emission charateristics of these anions. This investigation was intended to determine whether low emitter fluorescence efficiencies were limiting the light output of the reactions. Orthoacetamidobenzaldehyde (AAB) was included in the study. This compound is the analogous oxidation product from the parent heterocyclic compound, indole, which has a very low chemiluminescent output.

Figure 23 depicts the absorption spectra of the product anions. The absorption spectra were independent of the base-to-compound ratios (10-1000) employed in these luminescence measurements. In some cases, AAB proved to be unstable in basic solution, necessitating the determination of the absorption and luminescence spectra (when fluorescein was standard) as a function of the time of mixing. The optical density was then corrected to zero time. This resulted in a correction of about 10%.

The luminescence quantum yield measurements were made relative to fluorescein (0.1N NaOH) and quinine sulfate (0.1N H_2SO_4). The optical densities of the reference solutions and the anions were fixed at 0.020 at the excitation wavelength. Corrections were applied for the different refractive indices of the standards, n = 1.33 for water and 0.1N sulfuric acid, and n = 1.44 for DMSO. The quantum efficiency of fluorescence of fluorescein was taken as 0.80 for both wavelengths used, and 0.55 for quinine sulfate (ref. 11). The results are summarized in Table 14.

Table 14

LUMINESCENCE EFFICIENCIES OF THE ACYLAMIDE ANIONS DERIVED FROM SEVERAL INDOLES

	Fluorescein Standard[d]		Quinine Sulfate Standard[c]		
Compound[a]	Exciting Wavelength (mμ)	Degassed[b] Solution	Exciting Wavelength (mμ)	Degassed[b] Solution	Air Saturated Solution
FAP	365	0.20	365	0.23	0.27
AAP	365	0.46	365	0.15	0.078
AAB	435	0.05	365	0.097	0.021

a) Optical density of all solutions was 0.020 at the excitation wavelength.
b) Nitrogen purged solution.
c) Twice recrystallized from ethanol.
d) Used as received.

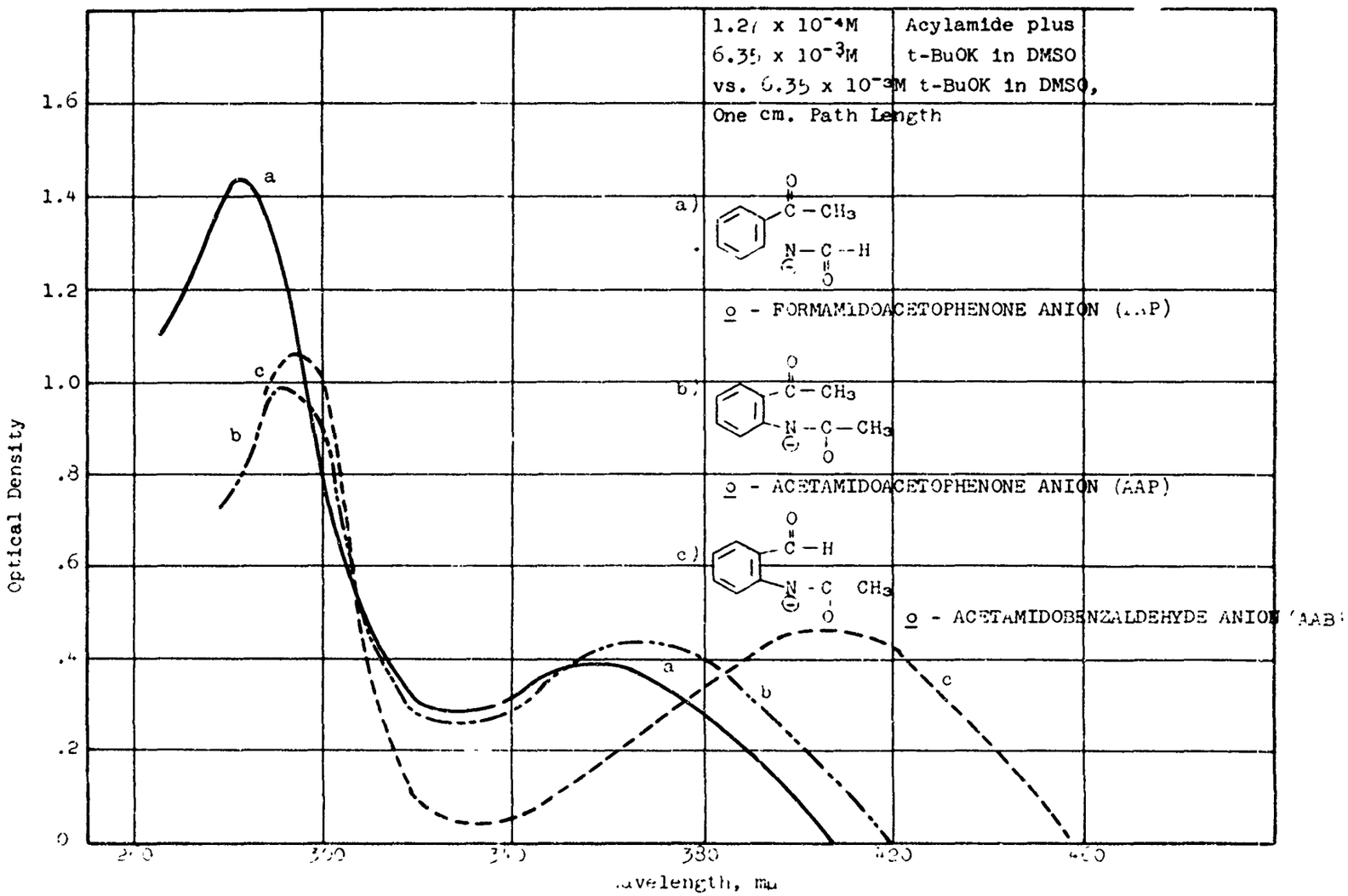


Figure 23. Absorption Spectra of Acylamide Anions

The intensity of the luminescence emitted from AAB when quinine sulfate was used as reference did not change with time as might have been expected from the decay of the AAB absorption spectrum. The reason for this is not known, but may involve impurities in the reagents used. The results obtained for the two standards are essentially the same. However, the results using the fluorescein standard are considered more approximate since the standard material was used without purification. In addition, the absorption spectrum of fluorescein is at a minimum at 365 mμ where the presence of trace impurities could alter the results.

A decrease in quantum yield due to the presence of oxygen is observed for the anions of AAB and AAP. Taking the rate constant of diffusion of oxygen to be approximately 5×10^9 1/mole-sec, the life time of the excited state is calculated to be of the order of 10^{-6} seconds. Since the anion of ortho formidoacetophenone is not quenched by oxygen, the lifetime of this transition is shorter than 10^{-6} seconds.

The emission lifetime is probably related to the nature of the optical transition involved. The absorption spectra of the three acylamide anions suggest that the long wavelength transitions correspond to those of an aryl carbonyl. The presence of a hydrogen on this carbonyl (AAB vs AAP)produces a red shift of the long wavelength band, $\lambda_{AAB}-\lambda_{AAP}$ = 35 mμ or 6.5 Kcal, as compared to the small blue shift for the introduction of hydrogen at the amide carbonyl, $\lambda_{FAP}-\lambda_{AAP}$ = -13 mμ which is equivalent to 2.8 Kcal.

In carbonyl compounds, the $n\rightarrow\pi^*$ transition is of lower energy than the $\pi\rightarrow\pi^*$ transition. The assignment of the long wavelength transition to the carbonyl group is consistent with the observed hypsochromic effect of methyl substitution. The electron-release effect of methyl raises the energy level of π^* state but does not appreciably affect the n lone pair orbital (ref. 19).

The effect of methyl substitution on the excited state appears to be related to the -N- C=O linkage. It is possible that the nature of the charge distribution and geometry of the excited states are modified by methyl substitution. The extinction coefficients of the long wavelength transitions do not reflect the differences in the lifetimes and quantum yields observed. The interpretation of fluorescence spectra is less certain than that of absorption spectra, since the knowledge of radiationless transitions and intersystem crossing,which is necesary, is generally unknown. Because a second emission peak was not observed degassed solutions, there may be no phosphorescence contribution to that total emission. The measured lifetimes of the excited state, lies in the usually observed range of 10^{-9} to 10^{-6} seconds for fluorescence emission.

The strong effect of a relatively weak electron donating methyl group demonstrates the importance of substitution effects. With extreme substituent effects, the lifetime of the excited state

may be sufficiently lengthened so that quenching may be complete. In air-saturated solutions, the luminescence quantum yield of the anion of AAB is at least a factor of ten less than that of the anion of FAP.

The very low chemiluminescence brightness and low efficiency of 2-methylindole is an indication of the importance of the chemistry (side-reactions) of the system, since the fluorescence efficiency of the product is only four times less than that for skatole.

E. QUANTUM YIELD OF SKATOLE CHEMILUMINESCENCE

1. General

The determination of the quantum efficiency requires the measurement of the total photon emission produced during (1) the consumption of a known number of moles of reactant, or (2) the formation of a known amount of product. A product analysis was made by independent methods, spectrophotometry and fluorometry.

2. Spectrophotometric Method

In the spectrophotometric method, a very dilute solution (3.1×10^{-4}M) of t-BuOK in DMSO was used in order to reduce the effects of discoloration of the solution during oxidation. The autoxidation was continued until the photometric signal was less than one per cent of the peak intensity. A known volume of water was then added to neutralize both the reaction product and any unreacted indole anion. The absorption spectrum of this type of solution is shown as curve a in Figure 24 together with the calculated spectra (Band c) for pure DMSO, assuming that only skatole and FAP are present. The sum of the concentrations from Band c equals the initial concentration of skatole before oxidation.

The calculated curve, (the sum of curves b and c) does not precisely fit the experimental curve, a. The effect of water on the absorption spectrum was determined with 1.26×10^{-4}M FAP and 0.96×10^{-4}M skatole in a 5:2 DMSO-H_2O solvent mixture. This was measured to determine the extent of solvent shifts between DMSO and DMSO-H_2O solutions. The discrepancies between the spectrum of this synthetic solution and the experimental one may be due to the presence of other products of skatole oxidation in the experimental solution or to the discoloration of DMSO by base in the oxidized solution. However, the matching of the curves above 320 mμ, and the presence of a plateau at 320 mμ, shows that FAP is present in the oxidized solution in at least 55 mole-% of the initial concentration of skatole. The plateau at 280 to 290 mμ indicates an unresolved absorption band that is most likely due to unoxidized skatole.

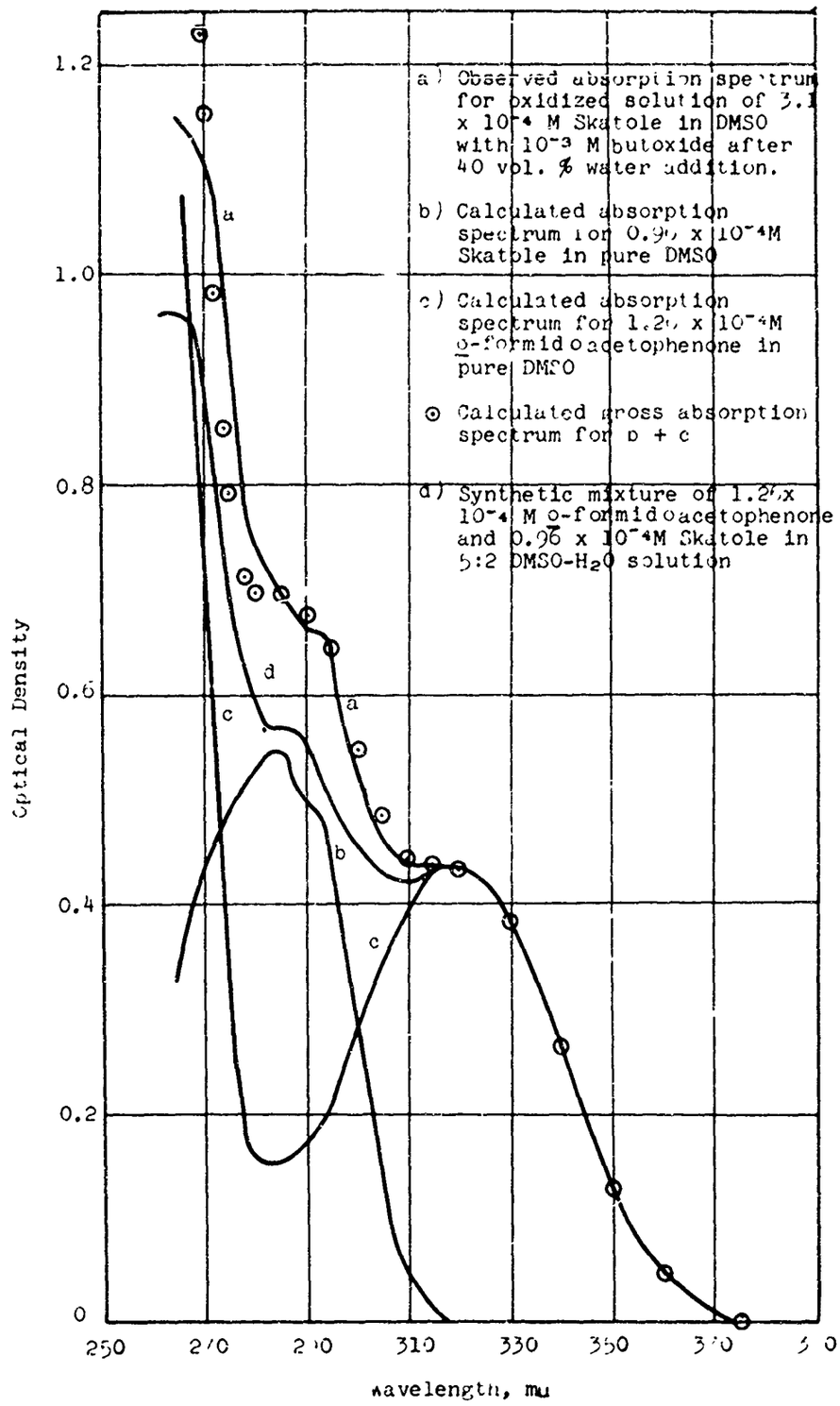


Figure 24. Absorption Spectrum of an Oxidized Skatole Solution

The total light emission was measured with the spectrophotometer. The sensitivity of our spectrometric-photomultiplier (including optics) was determined in fixed geometry by reference to luminol autoxidation in DMSO, for which Lee and Seliger's careful standardization is available (ref. 12). The emission from this system was recorded as a function of time at fixed bandwidth (about 1 mμ) at the 484 mμ emission peak, and integrated to obtain the response in coulombs per molecule of luminol decomposed per cubic centimeter. A similar measurement was made at the same wavelength and bandwidth for the skatole system. Determination of the chemiluminescence spectrum of skatole as a function of decay time revealed no change in the relative intensity distribution. From the known spectral distributions of both systems, the relative photon emission integrated over wavelength and time is obtained. By comparison to the luminol data, the total photon emission per mole of skatole per cm^3 is found.

For a 3.1×10^{-4}M skatole solution, 7.7×10^{13} photons/cm^3 are emitted during the autoxidation. On the basis of the FAP product yield, the quantum efficiency is 0.11%. Since the skatole consumption is between 55 and 100%, the overall chemiluminescence yield is between 0.05 and 0.1%.

3. Fluorometric Method

The second procedure combined photometric and fluorometric techniques. The chemical yield of FAP was determined by the measurement of the fluorescence intensity of an oxidized solution of skatole. No emission corresponding to the unreacted skatole anion was observed in the luminescence spectrum of the oxidized solution. A calibration curve was made of the fluorescence intensity emitted from solutions of known concentrations of FAP at base concentrations identical to those in the autoxidation experiments. The photometer, calibrated with a luminol solution (10^{-3}M t-BuOK, 10^{-6}M luminol in DMSO), measured the emission at all spectral wavelengths. Since luminol and skatole have chemiluminescence emission peaks at similar wavelengths (484 mμ and 495 mμ), a correction for the spectral sensitivity of the 1P28 photomultiplier would be negligible and was not included in the calculations. The results of the two methods are summarized in Table 15.

TABLE 15

PHOTOMETRIC AND FLUOROMETRIC SPECTROMETRY OF SKATOLE

Skatole (M)	Base (M)	Chemical Yield of FAP, %	Chemiluminescence Quantum Yield, %
3.1×10^{-4}	10^{-3}	ca.55	0.11*
2×10^{-4}	10^{-2}	42	0.12†
2×10^{-4}	5×10^{-3}	23	0.24†

* Determined by Absorption Spectrometry
† Determined by Fluorometry

From the measured fluorescence yield of FAP (Table 14), the excited state yield (on a product basis) is 0.5%. It is concluded, then, that over 99% of the FAP produced is formed directly by dark reaction, i.e., either directly in the ground state or as excited triplet that is nonradiatively quenched.

The possibility of intermolecular quenching by skatole or its major reaction product is ruled out by the non-dependence of the chemiluminescence efficiency on skatole concentration (at low concentrations), and by experiments in which FAP was added to the initial reaction mixtures without effect. Base quenching may similarly be excluded.

F. THE MECHANISM OF SKATOLE CHEMILUMINESCENCE

The autoxidation of skatole in alkaline solution was shown in the previous section to proceed with high (25-50%) chemical efficiency. Chemiluminescence is not observed if a solution of skatole and perbenzoic acid in DMSO is heated to 100°C in the absence of base. The need for a basic medium was also observed in the aqueous persulfate skatole oxidations (ref. 5). That the anion of skatole plays an important role in the oxidation of this compound is shown by the fact that the maximum intensity of chemiluminescence levels off when the base concentration is approximately equal to the concentration of skatole (Figure 14). Consistent with this fact are the results of some qualitative experiments performed with approximately 10^{-4}M skatole and 10^{-2} to 10^{-1}molar t-BuOK. A neutral solution of skatole was exposed to 3000 to 4000Å radiation, and showed, as expected, no visible fluorescence. Injection of the base produced an immediate blue fluorescence, which did not change with time or the addition of more base. Hence, in excess base, skatole is converted to its anionic form within the time required for mixing (2 seconds).

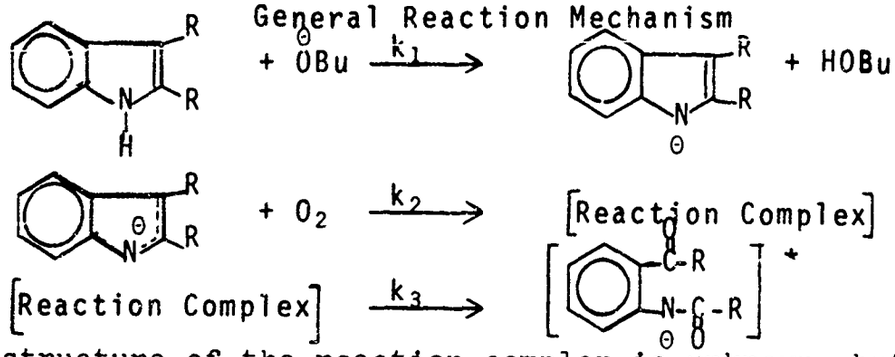
The addition of ortho-formidoacetophenone (FAP) prior to initiation of oxidation affects neither the total light yield nor the kinetics of the chemiluminescence. Thus FAP-sensitized emission does not occur in this system. Since the chemiluminescence emission spectrum observed in 5×10^{-2}M base and 3×10^{-4}M skatole solution is independent of the extent of oxidation, only one emitting species is involved.

The detailed nature of the reaction steps following the initial formation of the anion of skatole is uncertain. The reproducibility of the chemiluminescence emission curves is not high enough to permit unambiguous conclusions concerning the detailed mechanism of chemiluminescence. Reproducibility difficulties may be related to the age of t-BuOK-DMSO solution, and the efficiency of oxygen saturation. Base solutions turn

yellow on standing, at an unpredictable rate.

A possible general reaction scheme that is consistent with the requirements of the chemiluminescent reaction is the following:

General Reaction Mechanism

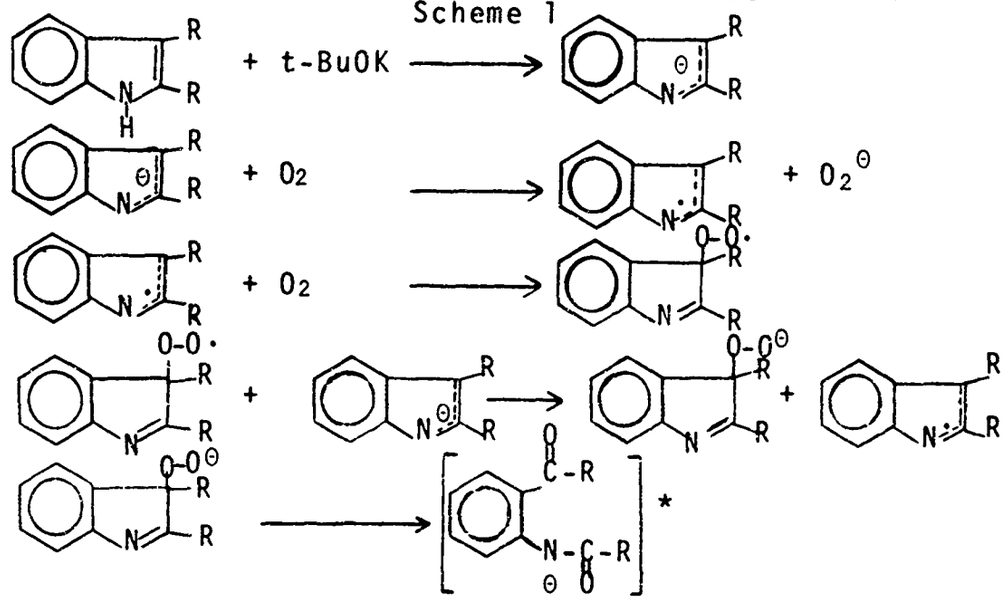


The structure of the reaction complex is unknown, but a peroxide or hydroperoxide is a logical intermediate.

The reaction of similar anions (e.g., RMgX, RLi) with molecular oxygen has been reported (ref. 13). These salts are covalent in character; this property may contribute to the lowering of the activation energy of the oxygen addition reaction. The base-promoted autoxidation of 2,4,6-tri-(t-butyl)phenol is catalyzed by compounds that can form an election donor-acceptor complex (ref. 14).

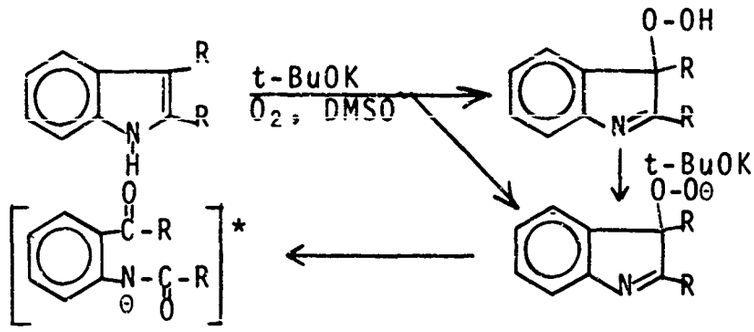
Several more specific mechanisms are suggested by the data:

Scheme 1



The base-induced decomposition of 2,3-dimethylindole hydroperoxide has been shown to lead to ortho-acetamidoacetophenone (ref. 15).

Scheme 2



In the mechanisms proposed above, the key intermediate is assumed to be a hydroperoxide anion. Since oxygen has a triplet ground state, the possibility exists that the excited state of the emittor may be in a triplet rather than a singlet state. A triplet intermediate could explain the low chemiluminescence quantum yield. If a triplet intermediate is involved, it must be a second or higher electronically excited state, since the luminescence of the ortho-formamidoacetophenone anion has been shown (in a previous section) to be fluorescence emission, i.e., emission from the first excited singlet state. Internal conversion and intersystem crossing would have to occur before the first excited singlet state was reached from any higher triplet state. Since intersystem crossing (spin inversion) is an unprobable transition, a triplet intermediate could lead to low chemiluminescence efficiencies.

A peroxy radical scavenger, β-carotene, was added to a reaction mixture to determine the importance of radical intermediates.

A saturated solution of β-carotene in DMSO was introduced at the moment the intensity of the chemiluminescence reached its peak. The light intensity was observed to decrease immediately by about 50%. This result was found for two different β-carotene injection rates at equivalent added concentrations of the scavenger. A fraction of the reduction in light yield is due to absorption of the emission by β-carotene. The red color of β-carotene was not discharged during the oxidation, and the chemiluminescence intensity did not increase when the scavenger flow was stopped. Similar experiments were performed with potassium iodide, sodium thiosulfate, and potassium cyanide additives. No decrease in chemiluminescence was observed. However, the addition of iodine, potassium periodate, or copper sulfate quenched the light emission. Therefore, the test for hydroperoxide is inconclusive.

While the details of the individual reaction steps cannot be described with certainty based on the above data, a logical reaction path from skatole to excited o-formamidoacetophenone can be devised. This is shown in Figure 25.

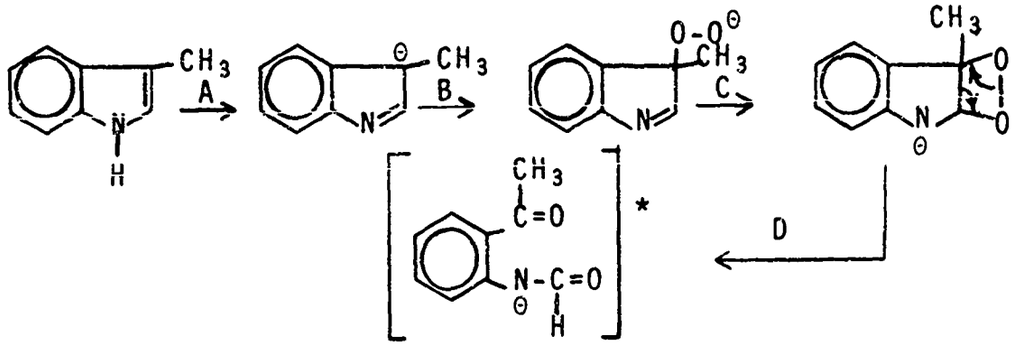


Figure 25. Possible Reaction Mechanism for Skatole Oxidative Chemiluminescence

Several reaction steps -- free radical, ionic, or both -- may complicate step B. Several unsuccessful attempts to prepare a hydroperoxide derivative of 2,3-dimethylindole for testing as a chemiluminescent intermediate were made (Appendix I).

G. INDOLE SUBSTITUENT EFFECTS

Substituents were found to strongly affect the chemiluminescent properties of the more than forty indole derivatives studied. The effect on the light peak intensity and overall light yield was studied as a function of substituents on the benzene and heterocyclic rings. The data are presented in Tables 16, 17, and 18. Table 19 contains the order observed for the integrated light output.

The absence of light emission from N-methylindole suggests that abstraction of the amine proton by the butoxide anion triggers the oxidation reaction. The ease of formation of the anion, the stability of the anion to electron-transfer reactions, and the fluorescence efficiency of the excited state are influenced by the addition of functional groups to various parts of the reacting molecule.

A comprehensive interpretation is difficult since neither the product yield of each indole nor the quantum yield of fluorescence of the emittor has been determined. Within these limitations, several qualitative conclusions may be drawn from the data in Tables 16-19.

Substitution in the 3-position is a necessary condition for bright chemiluminescence. Within this class of derivatives, different functional groups give a wide variation in results. Non-conjugative electron donors (e.g., alkyl groups) provide the brightest indole derivatives. These groups may either destabilize a 3-anion and make it very reactive, or may stabilize a 3-radical, increasing the probability of its formation. Electron-withdrawing groups (e.g., carbonyl or olefinic groups) that decrease the anion or radical density at the 3-position (XI) decrease the

Table 16

CHEMILUMINESCENCE OF INDOLES WITH SUBSTITUTED BENZENE MOIETY

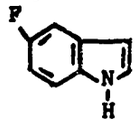
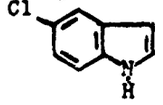
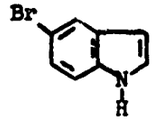
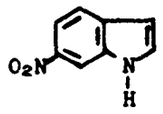
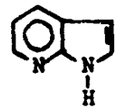
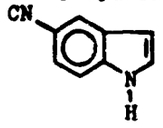
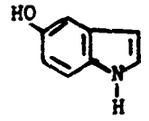
Compound and Structure	Peak O_2 Current I/I_0	Time to O_2 Peak sec	$T_{1/2}$ of O_2 Peak sec	Figure of Merit
5-Fluoroindole 	6×10^{-3}	70	200	1.2
5-Chloroindole 	$(2.6 \pm 1.0)10^{-2}$	53	720±60	18.7
5-Bromoindole 	$(1.7 \pm 0.3)10^{-2}$	255	6.4×10^3	88
6-Nitroindole 	2×10^{-4}	15	18	3.6×10^{-3}
7-Azaindole 	$(4.8 \pm 0.6)10^{-2}$	66	$(9.1 \pm 1.2)10^3$	4.4×10^2
5-Cyanoindole 	2.6×10^{-2}	-	3.2×10^4	8.4×10^2
5-Hydroxyindole 	2.6×10^{-3}	360	1.5×10^3	3.9

Table 16
(Continued)

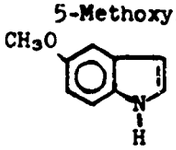
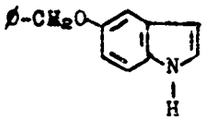
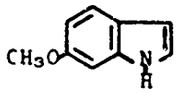
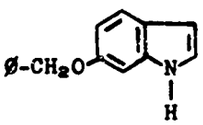
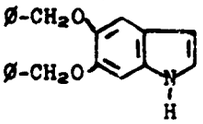
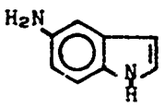
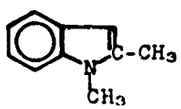
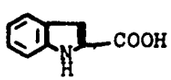
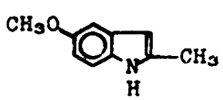
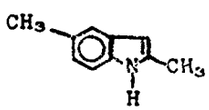
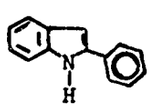
Compound and Structure	Peak O_2 Current I/I_o	Time to O_2 Peak sec	$T_{1/2}$ of O_2 Peak sec	Figure of Merit
5-Methoxyindole 	1.3×10^{-2}	-	120	1.6
5-Benzyloxyindole 	0.1	40	600	60
6-Methoxyindole 	0.5	-	$(6.4\pm1.4)10^{-3}$	$(3.2\pm0.8)10^{3}$
6-Benzyloxyindole 	0.75 ± 0.10	-	$(5.3\pm2.1)10^{3}$	$(4.4\pm1.4)10^{3}$
5,6-Dibenzyloxyindole 	0.38	40	1260	430
5-Aminoindole 	1.1	-	13	1.4

Table 17

INDOLES WITH SUBSTITUENTS ON THE PYROLE MOIETY

Compound and Structure	Peak Brightness I/Io	Time to Peak Emission sec	$T_{1/2}$ sec	Figure of Merit	Comment[1]
		a) 2-Indoles			
1,2-Dimethyl indole 	1.2×10^{-2}		270	3.2	99.7%
Indole-2-carboxylic acid 	5×10^{-3}		300	1.5	
5-methoxy-2-methyl indole 	1×10^{-2}		360	3.6	
2,5-dimethyl indole 	0.89 ± 0.2	40	178 ± 28	153 ± 9	98.4%
2-phenyl indole 	2.4×10^{-3}	440	665	1.6	

[1] Purities, where determined, are approximate mole-% as found by VPC analysis. The results for the substituted indole-3-acetic acids are based upon the observed distributions of decarboxylated products.

Table 17 (Continued)

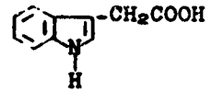
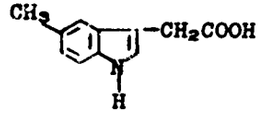
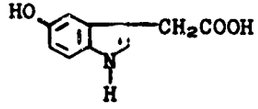
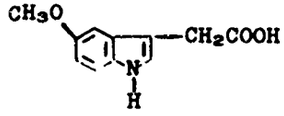
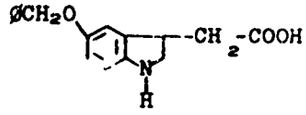
Compound and Structure	Peak Brightness I/Io	Time to Peak Emission sec	$T_{1/2}$ sec	Figure of Merit	Comment
		b) 3-indoles			
Indole 3-acetic acid 	9.1 ± 5.5	60	235 ± 15	2.1 x 10^3	
5-Methylindole-3-acetic acid 	24.6 ± 10.2	20	81 ± 21	(1.8 ± 0.3)10^3	85%
5-Hydroxyindole-3-acetic acid 	2 x 10^{-3}	48	240	0.5	
5-Methoxyindole-3-acetic acid 	45.2 ± 5.2	25 ± 3	99 ± 3	(4.5 ± 0.7)10^3	97%
5-Benzyloxyindole-3-acetic acid 	41 ± 5	42 ± 3	152 ± 28	(6.1 ± 0.4)10^3	97%

Table 17 (Continued)

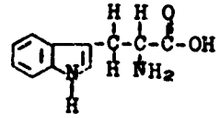
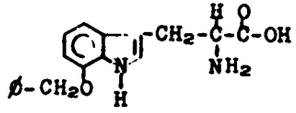
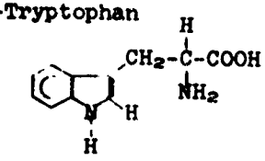
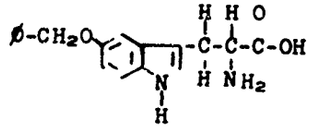
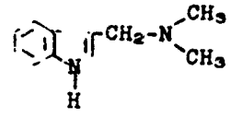
Compound and Structure	Peak Brightness I/Io	Time to Peak Emission sec	$T_{1/2}$ sec	Figure of Merit	Comment
5-Hydroxytryptophan 	$2.6(10)^{-3}$	30	180	0.47	
7-Benzyloxytryptophan 	$7(10)^{-2}$	30	900	63	saturated solution
L-Tryptophan 	.86	-	483	440	
5-Benzyloxytryptophan 	1.35 ± 0.05	60 ± 0	450 ± 60	605 ± 58	
Grammine 	0.11 ± 0.02	28	110 ± 70	12.1	

Table 17 (Continued)

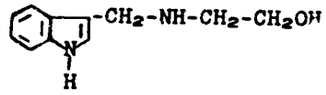
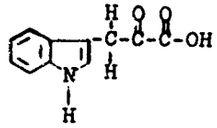
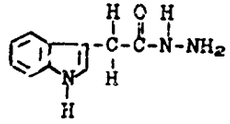
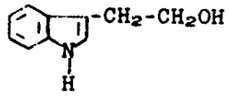
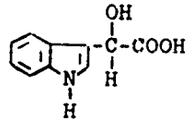
Compound and Structure	Peak Brightness I/Io	Time to Peak Emission sec	T 1/2 sec	Figure of Merit	Comment
(3-Indoylmethylamino)ethanol 	0.9×10^{-2}	20	48 ± 2.5	0.43	
Indole-3-pyruvic acid 	0.35 ± 0.06	15 ± 3	3.3 ± 0.3	1.15 ± 0.28	
Indole-3-acetic acid hydrazide 	1.18 ± 0.1	57	168 ± 12	197 ± 3	
Tryptophol 	0.76	70	450	342	
Indoleglycolic acid 	1.9×10^{-2}	36	276	5.2	

Table 17 (Continued)

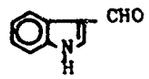
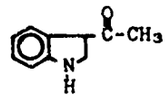
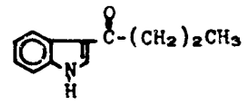
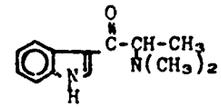
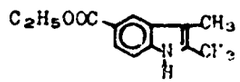
Compound and Structure	Peak Brightness I/I_o	Time to Peak Emission sec	$T_{1/2}$ sec	Figure of Merit	Comment
Indole-3-carboxaldehyde 	4.6×10^{-2}	60	300	13.8	
3-Acetylindole 	$(3.0 \pm 0.1)10^{-2}$	92 ± 12	321 ± 21	9.6	
3-Butyrylindole 	1.4×10^{-2}	12	48	0.67	
(β- Dimethylamino-propionyl)indole 	1.3 ± 0.3	65	600	7.8	
c) 2,3-Disubstituted Indoles					
Ethyl 2,3-dimethyl indole-5-carboxylate 	8	--	520	4160	100.0%

Table 17 (Continued)

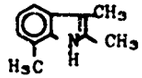
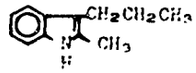
Compound and Structure	Peak Brightness I/Io	Time to Peak Emission sec	T 1/2 sec	Figure of Merit	Comments
2,3-Dimethylindole-5-carboxylic acid	156 ± 4	18 ± 3	48 ± 6	7540 ± 820	Solvents purged with N_2.
Same as above	111 ± 11	31 ± 11	42 ± 2	4710 ± 210	Solutions air saturated.
2,3,7-Trimethylindole 	14.4 ± 3.3	47	67 ± 9	965	97%
5-Amino-2,3-Dimethylindole	4.3 ± 1.7	8	18 ± 4	68 ± 1.0	
2,3-Dimethyl-5-nitroindole	not detected	--	--	--	
2-Methyl-3-propylindole 	6.7 ± 0.7	14	40 ± 2	266 ± 14	Crude product; ~50%

Table 17 (Continued)

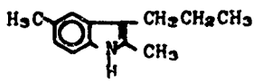
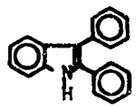
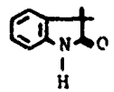
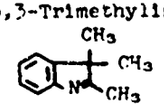
Compound and Structure	Peak Brightness I/I_0	Time to Peak Emission sec	T 1/2 sec	Figure of Merit	Comments
2,5-Dimethyl-3-propylindole 	17.2 ± 1.2	10	24 ± 0	413 ± 29	Crude product ≈46%
2,3-Diphenylindole 	$(2.1\pm0.7)10^{-3}$	≈10	≈15	3.2×10^{-2}	
d) Indole Derivatives					
Indoline	0.4 ± 0.05	145	745 ± 35	298	
Oxindole 	0.19	20	1	--	
2,3,3-Trimethylindolenine 	0.83 ± 0.21	7 ± 2	27 ± 3	22 ± 4.5	

Table 18

CHEMILUMINESCENCE OF MONOSUBSTITUTED ALKYL-INDOLES IN DMSO

Indole Concentration: 5×10^{-3}M
t-BuOK Concentration: $>5 \times 10^{-2}$M

Compound	Purity	% Skatole	100 I/I_0 Crude	100 I/I_0 Corrected[a]	$t_{1/2}$, seconds	Figure of Merit, seconds, Corrected[a] $I/I_0 \times t_{1/2}$	Comments
Indole	99.7	0.3	30	5	250	13	Purified sample in preparation.
1-Methylindole	90.1	0.5	38	(≈0)	(450)	(≈0)	Major unidentified impurity ≈10%.
2-Methylindole*	100	0.0	6	6	54	3.2	High purity.
3-Methylindole	99.9	-	8.4×10^3	8.4×10^3	308	2.6×10^4	Averaged values: three impurity peaks.
3-Methylindole	99.98[b]	-	8.9×10^3	8.9×10^3	363	3.2×10^4	
4-Methylindole	89.4	0.0	1.3	1.3	(2.3×10^3)	30	No skatole, major unidentified impurity ≈10%.
5-Methylindole	100	0.0	30	30	3.3×10^3	10^5	High purity.
6-Methylindole	95.6	0.35	80 ± 30	50 ± 30	$(3.1 \pm 1.4) \times 10^3$	$(1.9 \pm 1.4) \times 10^5$	Average of six runs, highly variable, ten impurity peaks. Average of four runs for figure of merit.
7-Methylindole	99.0	?	40	40	360	140	Two unidentified peaks, skatole masked by major product.
3-Ethylindole	99.8	-	2×10^3	-	660	1.3×10^4	Comparable to skatole in brightness and efficiency.

* Previously reported.

a Peak brightness corrected for skatole impurity assuming molar additivity at observed peak.

b Zone refined sample.

Table 19

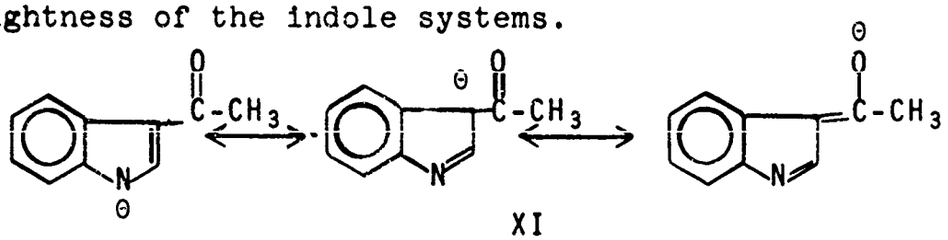
TABULATION OF BRIGHTER CHEMILUMINESCENT INDOLE DERIVATIVES -- IN ORDER OF FIGURE-OF-MERIT

	Compound	$FM^{\dagger} \times 10^{-3}$
1.	3-Methylindole	26
2.	3-Ethylindole	13
3.	2,3-Dimethylindole-5-carboxylic acid	6
4.	5-Benzyloxyindole-3-acetic acid	6
5.	5-Methoxyindole-3-acetic acid	4.5
6.	6-Benzyloxyindole*	4.4
7.	Ethyl-2,3-dimethylindole-5-carboxylate	4.2
8.	Indole-5-carboxylic acid*	3.8
9.	2,3-Dimethylindole	3.6
10.	6-Methoxyindole*	3.2

* Compounds with average emission decay times of the order of one hour. With the exception of indole-5-carboxylic acid, individual decay times are highly irreproducible.

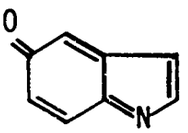
† Figure of Merit (see Definitions and Abbreviations)

brightness of the indole systems.



XI

Although a quantitative analysis of substituent effects is not feasible, since most compounds of this group are of unknown purity, the qualitative effects are consistent for the brighter species. Thus, 5- and 6-substitution by methyl, methoxy and benzyloxy radicals increases the chemiluminescence efficiency of indole-3 acetic acid, as does 5-carboxyl substitution. Similar effects are observed in tryptophan. On the other hand, 5-substitution of amino or hydroxyl groups leads to marked reduction in efficiency, probably through the formation of XII in a competitive side reaction.



XII

Besides influencing the course of the chemical oxidation, substituents will influence the fluorescence efficiency of the excited intermediate. In general, electron donating groups increase fluorescence efficiencies while with-drawing groups have the opposite effect (ref. 51). In a previous section, it was shown that the placement of methyl groups on the carbonyl groups (equivalent to 2- and 3-substitution on indole) has a striking effect on yield and lifetime of the excited state.

As a result of the promising data for 2,3-dimethylindole-5-carboxylic acid, several 5-substituted skatole derivatives were synthesized (see Appendix I). The compounds are odorless. The chemiluminescence parameters for these derivatives are given in Table 20. No improvement in chemiluminescence efficiency compared to skatole is observed. The variations in reaction rates, attributed to the age of the base solution, had little effect on the light yield.

H. THE LIGHT CAPACITY OF THE SKATOLE SYSTEM

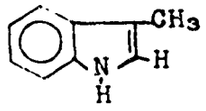
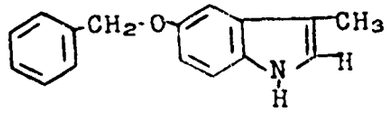
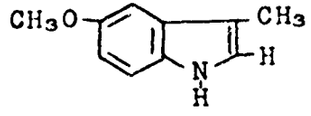
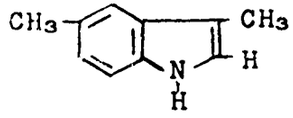
The light capacity (L), an integrated emission value suggested by the American Cyanamide Company, has been computed for the skatole system. The light capacity of a system is given by the following equation:

$$L = 4.07 \times 10^4 \, QMP$$

Q = Quantum yield, einsteins/mole

Table 20

CHEMILUMINESCENCE OF SKATOLE DERIVATIVES

Compound	Structure	$t_{1/2}$, sec	I/I_0	FMx10^{-4}*	$\int$FMx10^{-4}†	Remarks
Skatole		294±34	70±5	2.0±0.1	3.6±0.1	Aged base**
5-Benzyloxy Skatole		160±25	142±9	2.3±0.4	-	Fresh base
		275±35	68±7	1.9±0.3		Aged base
5-Methoxy Skatole		215±80	113±11	2.3±0.7	3.6±0.3	Fresh base
5-Methyl Skatole		184	81	1.5	2.6	Aged base

* FM is the previously defined figure-of-merit, $I/I_0 \times t_{1/2}$ (sec).

† The $\int$FM is the directly recorded integrated photometer current in coulombs divided by the photometer current produced by the standard source (to permit comparison to FM).

** Aged base was used about 24 hours after preparation.

M = Concentration, moles/liter

P = Phototropic efficiency

The phototropic efficiency (P), which is the extent of overlap of the chemiluminescence emission spectrum and the spectral sensitivity of the eye, was found to be 41% for the skatole system.

If L is calculated for the skatole concentration at which the quantum yield was determined, the values of the parameters are: $Q = 7.5 \times 10^{-4}$ einsteins/mole, P = 0.41 and $M = 3.1 \times 10^{-4}$M. The precise value for Q is uncertain since the amount of skatole undecomposed could not be determined quantitatively from the spectrometric analysis. If the average value between the two extremes of 50 and 100% decomposition is used in the equation, the value of L will be within 25% of its true value. L is calculated to be 3.9×10^{-3} lumen-hours per liter from the above values.

The maxima in light per mole and brightness, however, occur at the concentrations 2×10^{-2} skatole and 0.1M t-BuOK. The quantum efficiency (figure-of-merit) for skatole changes only slightly in the concentration range 3.1×10^{-4}M to 2×10^{-2}M (Figure 16). The light capacity, calculated for this optimum condition is 0.16 lumen-hours per liter.

I. CHEMILUMINESCENCE OF AQUEOUS SKATOLE SOLUTIONS

We conducted a short study to determine whether practical light emission levels could be obtained from the autoxidation of skatole in aqueous basic solutions. Our previous results showed that the skatole system is relatively bright in dimethylsulfoxide-potassium t-butoxide solution. An aqueous system with equivalent emission characteristics would have several advantages over the DMSO system for field use.

Solutions containing 9×10^{-5}M skatole and 0.2M sodium hydroxide in deionized water were screened with the oxidizing agents shown in Table 21. Light emission was not detected in any of these systems. This confirms the necessity for the use of non-aqueous solutions in skatole chemiluminescent reactions.

Table 21

AQUEOUS SKATOLE OXIDIZING AGENTS

(1) 0.6% H_2O_2
(2) 0.6% H_2O_2, 2.4×10^{-3}% NaOCl
(3) 0.6% H_2O_2, 5×10^{-5}M $K_3Fe(CN)_6$
(4) 5×10^{-5}M $K_3Fe(CN)_6$, O_2- saturated solution
(5) 3×10^{-2}g Hemoglobin in 25 ml test solution, O_2 - saturated solution

V. THE INFLUENCE OF SOLID SUBSTRATES ON CHEMILUMINESCENCE

A. INTRODUCTION

Many of the bright chemiluminescent systems are known to proceed through peroxide or hydroperoxide intermediates, with light emission postulated to be the result of the decomposition of these high energy compounds. Certain metals are known to accelerate autoxidation reactions vigorously. Similarly, peroxide decomposition catalysts are well known. We felt that chemiluminescent reactions, made to occur at suitable surfaces, might be more efficient than homogeneous solution reactions with respect to oxidation rates and fluorescence efficiencies. Also of value may be the bathochromic shift often associated with the adsorbed state of fluorescent materials.

B. PRELIMINARY RESULTS OBTAINED FROM THE OXIDATION OF LUCIGENIN AND LUMINOL ON SILICA GEL COATED ON GLASS SLIDES.

Several preliminary experiments were performed to develop a technique for the investigation of the effect of solid substrates on chemiluminescent reactions. A simple, qualitative procedure was used to measure chemiluminescence emission resulting from the oxidation of luminol and lucigenin catalyzed by silica gel G. Sheets of silica gel were prepared by coating the gel on glass slides. The slides were dipped in a water-silica gel slurry and were air-dried at room temperature. Methanol solutions of the organic compounds were sprayed from an atomizer onto the silica gel and dried. The base and oxidant (other than oxygen) were sprayed independently on the dry slides. The use of this technique produced a blue-green chemiluminescence from lucigenin (Table 22). The silica gel was wet during the course of the chemiluminescence, permitting the components (except for the solid substrate) to diffuse toward each other in the liquid phase. The decay of the light intensity was measured photometrically. The time required for the intensity to diminish to one-half of its value was about three minutes. The decay does not obey simple first order or second-order kinetics.

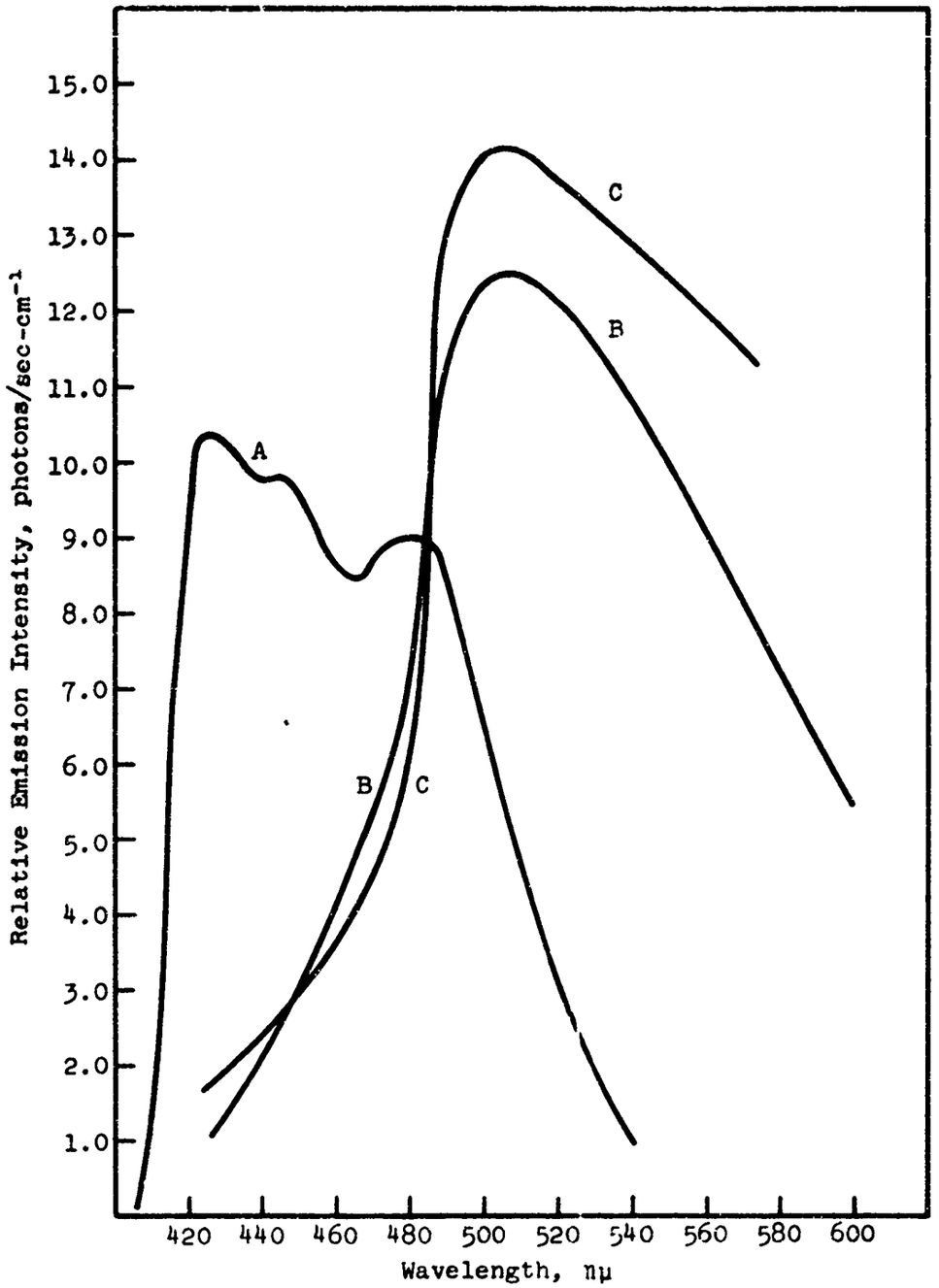
The chemiluminescence spectrum of the lucigenin oxidation in homogeneous ethanol solution is a broad, structured band between 420 mμ and 490 mμ (Figure 26). The emission from a silica gel surface is shifted to the red, giving a broad band peaking at 505 mμ. Similar emission spectra were obtained with either ammonium or sodium hydroxide as bases.

The oxidation of luminol by oxygen in t-BuOK-DMSO did not lead to chemiluminescence. Improvement of the techniques for measuring the luminol chemiluminescence on solid absorbants, (described in the following section) however, demonstrated that chemiluminescence can occur in the presense of silica gel.

Table 22

CHEMILUMINESCENT REACTION ON SILICA GEL ABSORBENT

Organic Compound	Base	Oxidant	Observation
Lucigenin	2M NaOH	10% H_2O_2 in ethanol	Blue-green chemiluminescence
Lucigenin	4M NaOH	10% H_2O_2 in ethanol	Decayed to one half peak intensity in 210 seconds
Luminol	0.05 to 0.1M K^+tBuO^- DMSO solution	O_2	No chemiluminescence



A = 10^{-3}M Lucigenin, 4% H_2O_2, 6.8M NH_4OH in Ethanol

B = Lucigenin, 2M NaOH, 30% H_2O_2 absorbed on Silica Gel G

C = Lucigenin, conc. NH_4OH, 30% H_2O_2 absorbed on Silica Gel G

Figure 26 Chemiluminescence Spec ra of Lucigenin in Ethanol Solution and on Silica Gel

C. PHOTOMETRIC MEASUREMENT OF CHEMILUMINESCENCE CATALYZED BY GAS-PERMEABLE SOLID MEMBRANES

1. Apparatus

Two apparatus were designed to measure the influence of solid substrate on chemiluminescence. The first design of the apparatus, Cell 1, is shown in Figure 27. This cell was later modified to take the form shown in Figure 28. The cell design provided the maximum opportunity for the oxidation to occur on the catalytic surface.

The vertical orientation of the photomultiplier with respect to the solution facilitated variation of the depth of the solution from zero to any convenient value. In this way self-absorption by the solution was minimized.

2. Solvents

The selection of the proper chemiluminescence reaction solvent is critical since the liquid solution must be capable of wetting the solid substrate without leaking through the membrane. DMSO was found to be a suitable general reaction solvent.

3. Membrane Supports

In other work, Monsanto Research Corporation developed a Teflon-bonded porous membrane as a rigid substrate for heterogeneous catalysis experiments. We selected this basic structure for our solid substrate studies.

4. Substrate Materials

The types of catalysts screened in the program can be separated into three classes: (1) metals, (2) solid supports, and (3) metals adsorbed on the supports. The noble metals platinum and palladium, together with cobalt and silver, were selected on the basis of their reported oxidative catalytic activity at low temperature. Silica gel, basic alumina, acetylene charcoal, and molecular sieve (5A) were investigated as solid supports in the presence and absence of the above metals.

5. Preparation of Catalytic Membranes

The procedures used for the fabrication of each membrane and for maintaining high catalytic activity were carefully standardized. The membranes were prepared by curing a catalyst-Teflon suspension at high temperature. A minimum suspension thickness of 0.020 inch is necessary to prevent leakage of liquid through the membrane. The electrocatalytic properties of the membranes prepared by these techniques has been demonstrated

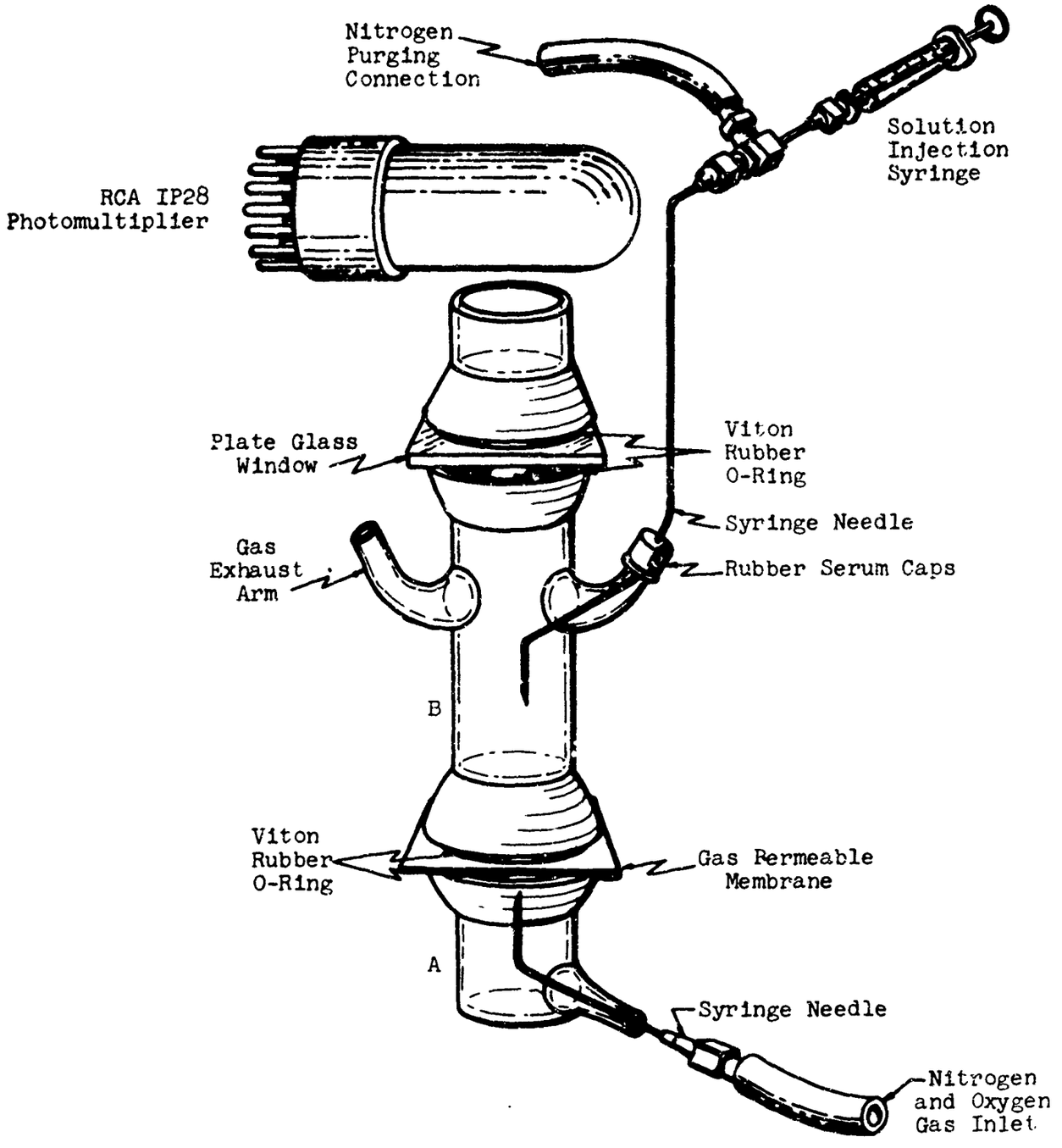


Figure 27. Heterogeneous Catalysis Apparatus, Cell 1.

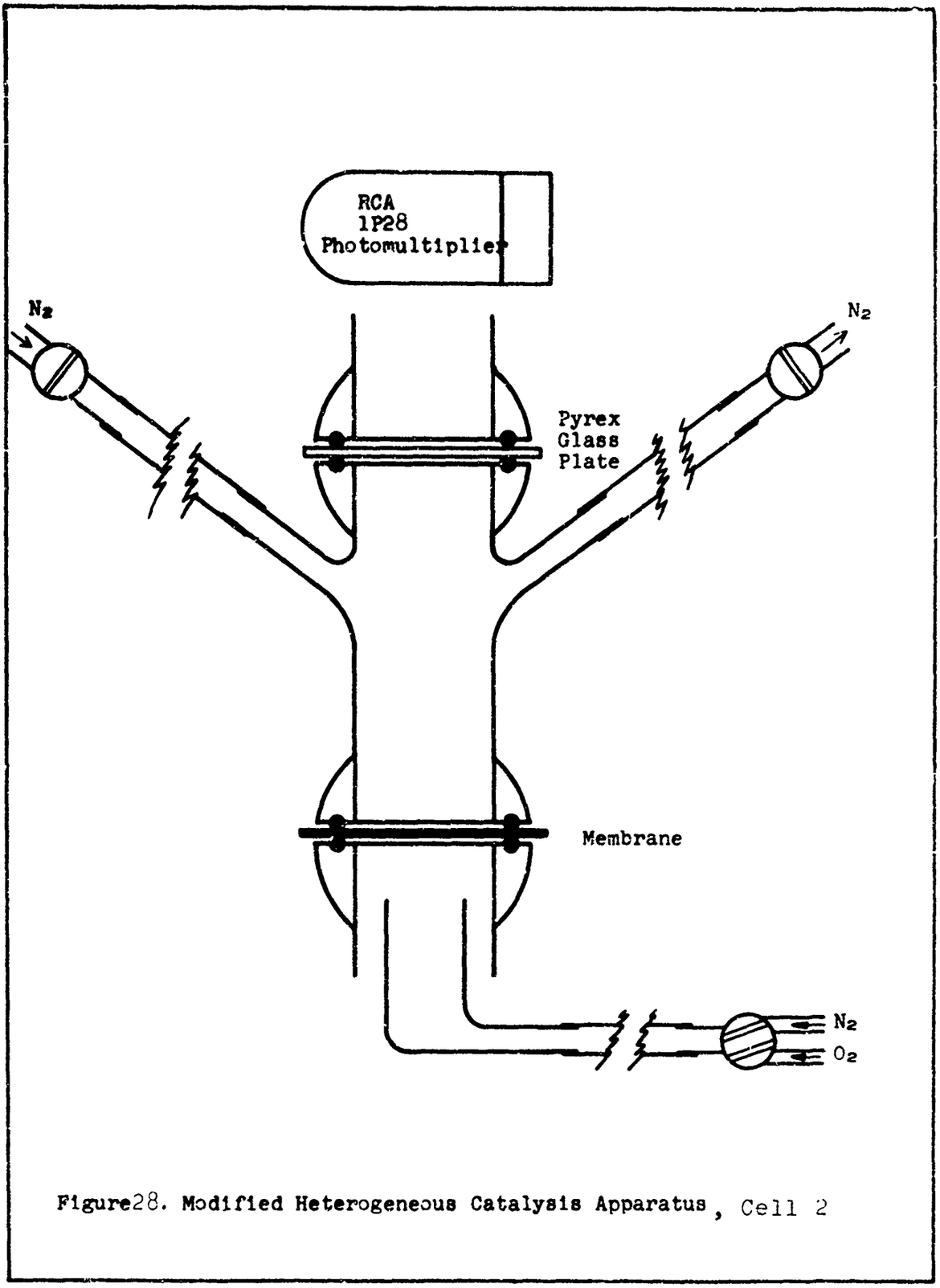


Figure28. Modified Heterogeneous Catalysis Apparatus, Cell 2

by their effectiveness as electrodes in exploratory electrochemical processes.

Silica gel and alumina become discolored when they are cured with Teflon above 200°C. This discoloration is possibly due to decomposition of absorbed material. The extent of darkening was minimized by curing the membranes at a maximum of 200°C.

The platinum-on-charcoal membranes were prepared by two methods. One procedure consisted of coating the absorbant (e.g., charcoal) with precipitated platinum from an aqueous chloroplatinic acid solution prior to incorporation of the mixture on a membrane. The second procedure consisted of precipitating platinum on a previously prepared membrane.

Two photometers were constructed to increase the number of experiments that could be run. To insure that the results obtained from the two photometers were directly comparable, the photometers were adjusted to respond similarly to a constant intensity light source. This was done by placing a blue tritium phosphor in the position normally occupied by the membranes in the test cell. The high voltage d-c power supply was adjusted so that both 1P28 photomultiphers produced the same current readings.

6. Experimental Procedure

Identical liquid volumes were employed in all runs. A 2-ml volume gave a solution depth of approximately 0.8 cm. The length of time the reagents were in contact with the membrane was standardized. The organic material to be oxidized was exposed to the membrane for three minutes prior to the injection of base. A second period of three minutes elapsed before oxygen was introduced into the lower chamber. In this way the results obtained for a membrane could be controlled and reproduced. Experiments were not performed to correlate contact times with chemiluminescence light yield.

The experimentally determined chemiluminescent decay curves did not correspond to a simple kinetic law. This precluded the application of an analytical method of integration to the determination of the light yield. The integrated light yield, therefore, was determined graphically.

D. RESULTS OBTAINED FROM THE OXIDATION OF LUCIGENIN AND SKATOLE

Several experiments were performed in t-BuOK/DMSO with skatole and lucigenin utilizing 5A molecular sieves and silica gel membranes. While no chemiluminescence was observed from the base-catalyzed auto-oxidation of lucigenin, the results obtained for skatole oxidation were promising. Cell 1, with a nitrogen purge through the chamber containing the DMSO solution,

was used for these experiments. At high oxygen pressures (Runs 1-3, Table 22a),oxidation probably occurred in the solution, since a measurable oxygen flow rate was detectable even after the gas had passed through the solution. The absence of any effect by an uncoated membrane was due either to the inertness of the solid or to homogeneous reactions predominating over heterogeneous processes.

At low oxygen pressures no bubbling was observed at the membrane surface. In this situation, oxygen diffused through the membrane at a rate where the possibility of reactions at the solid-liquid interface would be enhanced. The fivefold increase in light yield observed at lower oxygen pressure was later shown to be partially due to experimental factors. Because of this, the cell was modified to improve the reproducibility of the data. The chamber containing the liquid was isolated from the atmosphere with stopcocks (Cell 2, Figure 28). The skatole results using this cell are presented in Table 23.

The chemiluminescence of skatole in the presence of several membranes were measured. A Teflon membrane was used as a non-catalytic standard. It was assumed that the chemiluminescence observed in the presence of this membrane was characteristic of that from a homogeneous solution. Nc enhancement of the efficiency relative to the homogeneous solution was observed for the membranes tested.

E. INFLUENCE OF SOLID SUBSTRATES ON LUMINOL CHEMILUMINESCENCE

1. Preliminary Results and Reproducibility Experiments with Charcoal Membranes

The luminol chemiluminescent reaction was employed to test our experimental syatem for reproducibility. The results of the initial experiments (Table 24) indicated deviations ranging from 20% to 50% using Cell 1, which incorporated a nitrogen purge during the reaction.

The initial design was then modified in two ways. It was found necessary to increase the thickness of the membrane to reduce liquid leakage, and (as noted above) to isolate the liquid chamber to prevent agitation of the liquid. The new design markedly improved the reproducibility of the results. In experiments where more than one run was made, the percentage deviation from the average was $\pm$ 7%. Dry oxygen was introduced into the lower chamber at less than 50 ml/min (measured at 25°C and one atmosphere pressure).

Because of the ready availability of charcoal membranes with alumina backing, these membranes were used in the comparison of the light detection efficiency of our two photometers. The average values obtained from the two photometers were different

Table 22a

HETEROGENEOUS CATALYSIS OF SKATOLE CHEMILUMINESCENCE

5×10^{-4}M Skatole, 10^{-2}M Butoxide, Molecular Sieve 5A

Depth of Solution = 1.2 cm

Run No.	I_{max} 10^{6}amp	t_{max} 10^{-2}sec	$t_{1/2}$* 10^{2}sec	I_1† 10^{6}amp	τ_1† 10^{-2}sec	I_2† 10^{6}amp	τ_2† 10^{-2}sec	Figure of Merit‡ $(I_{max})(t_{1/2})+A$ 10^{4}amp-sec	Figure of Merit $I_1\tau_1+I_2\tau_2$ 10^{4}amp-sec	O_2†† Pressure or flow rate	Remarks
1.	3.9	1.86	2.1	-	-	-	-	13.4	-	0.04SCFH	Oxygen bubbles were dispersed in solution
2.	1.5	5.4	4.5	-	-	-	-	9.0	-	0.015SCFH	"
3.	1.1	5.2	5.4	-	-	-	-	8.0	-	0.015SCFH	"
4.	2.1	<0.1	-	0.57	7.2	1.53	27.0	-	45.4	3 inches of H_2O hydrostatic pressure	Teflon coated membrane without molecular sieve**, oxygen bubbles. No indication of oxygen bubbles in solution
5.	3.0	<0.1	-	1.87	3.0	1.14	34.8	-	45.2	"	"

* The decay of the chemiluminescence intensity obeyed first order kinetics and has a half life of $t_{1/2}$.

† The decay kinetics of the chemiluminescence intensity can be resolved into the sum of two first order processes having initial and final half lives of τ_1 and τ_2 respectively. I_1 and I_2 are the intensities obtained upon extrapolating each first order decay curve to zero reaction time.

‡ A represents a graphical integration of the light intensity from t=0, I=0 to $t=t_{max}$, $I=I_{max}$

†† SCFH is the rate of oxygen exhaust flow through the solution in units of standard cubic feet per hour.

** A membrane is coated on one side with the absorbant. The uncoated side was assembled in the apparatus facing the photometer so that the oxidized solution was in contact with a nonabsorbing surface.

Table 23

HETEROGENEOUS CATALYSIS OF SKATOLE CHEMILUMINESCENCE

(2.5 x 10^{-3}M Skatole, 2.5 x 10^{-2}M t-BuOK in DMSO)

Membrane	I_{max} 10^6 amp	Integrated Total Light Output amp-sec
Teflon	2.3	>127[b]
Alumina (basic)	3.3	79
Charcoal	1.5	73
Zinc Oxide	1.2	54
Talcum	0.75	32

a) Cell 2, with the liquid-containing chamber isolated from the atmosphere with stopcocks.

b) The oxidation was terminated when the intensity decayed to 5% of its maximum value. This occurred 5 hours after the initial introduction of oxygen.

Table 24

PRELIMINARY RESULTS FROM THE HETEROGENEOUS CATALYSIS OF LUMINOL

5 x 10^{-4}M Luminol, 1 x 10^{-2}M Potassium t-butoxide, Dimethyl Sulfoxide Solvent

Metal Catalyst	Solid Adsorbant	I_{max} (10^{-4} amp)	$t_{1/2}$ (sec)	Integrated Light* Output from I = 0 to I = I_{max}, Δ (amp-sec)	Integrated Light Output* of Decay Portion of Light Emission Curve $I_{max} \cdot t_{1/2}$, (amp-sec)	Total Integrated Light Output $I_{max} \cdot t_{1/2} + \Delta$ (amp-sec)	Average Total Integrated Light Output (amp-sec)	Remarks
None	Molecular Sieve 5A on Teflon	5.2	64	71	333	404		
		1.4	46	19	55	75		
		3.6	42	56	151	207		
		3.8	125	0	475	475	290 ± 160	
None	Teflon	3.3	55	64	116	180		The Teflon runs displayed erratic behavior in the decay curves
		2.5	100	0	250	250		
		3.8	113	50	429	479	303 ± 110	
Platinum black	Teflon	0.9	100	0	87	87		
Platinum slurry#	Teflon	1.1	142	15	156	171		Erratic decay
		1.6	48	51	75	126	149 ± 23	Erratic decay
None	Silica Gel on Teflon	0.9	100	12	113	125		Liquid leaked through membrane
		2.9	140	0	403	403		
Silver##	None	0.4	85	29	33	62		
Palladium	Teflon	0.2	385	0	61	61		
		0.5	405	2	20	22	42 ± 20	
None	Al_2O_3 on Teflon**	3.	35	0	105	105		
		4.0	140	0	560	560	414 ± 190	
		5.5	105	0	578	578		
None	Charcoal	3.0	105	0	330	330		
		1.2	175	0	215	215	273 ± 58	

*Determined by graphical integration.
†t1/2 is the time in which light emission intensity decays to one half of the maximum intensity.
#Flotronics metal filter; unactivated silver, 0.2 micron pore size.
##Platinum black was manually smeared on the Teflon.
**One side of the stainless steel screen was alumina bonded to Teflon, and on the other was charcoal bonded to Teflon.

However, the set of data from the two photometers overlapped (Table 25) at one-half of the standard mean deviation. Therefore, the two sets of data could be averaged, and the results obtained in later runs have not been distinguished with respect to the measuring photometer.

2. Effect of the Nature of the Catalyst on the Total Light Output

The influence of metals and activated absorbants can be assessed by comparing the photometric data obtained for these membranes to data obtained with an uncoated (and presumed inert) Teflon membrane.

As Table 25 shows, none of the catalyst systems screened increased the chemiluminescence efficiency of luminol. The observed integrated light outputs, which are a measure of the chemiluminescence efficiency, can be broadly classified into two groups. These groups consist of (1) outputs ranging from 55 to 230 amp-sec and outputs ranging from 360 to 610 amp-sec. Membranes with outputs in the former range are charcoal, platinum, palladium and cobalt; the latter range is represented by Teflon, molecular sieve 5A, basic alumina, and silica gel. These groupings correlate with the color of the membranes. The whiter membranes, with their higher reflectivities, produced the highest emission yield, while the black membranes give the lowest yields.

Within each grouping there is a wide variation of results. Platinum-on-charcoal membranes prepared by precipitating the metal on the absorbant prior to fabrication were 30 per cent better than charcoal membranes. This suggests that platinum may be exhibiting catalytic activity. However, the shapes of the light intensity decay curves follow irregular and erratic patterns. It is, therefore, difficult to abstract any information from these curves regarding catalytic activity. A palladium membrane produced lower light yield intensity than platinum membranes.

In the second category, Teflon, molecular sieve 5A, and silver were found to be approximately equal in efficiency. Since both Teflon and molecular sieve are considered inert supports, this finding suggests that silver is not active under these conditions. Alumina membranes produced 60% less light than did Teflon.

3. The Influence of Fluorescent Agents on the Chemiluminescence of Luminol

A large number of organic scintillators and dyes were screened for the following optimum properties: (a) color of the fluorescence emission, (b) stability in basic solution, (c) strong emission in alkaline medium, and (d) stability toward oxidation.

Table 25

HETEROGENEOUS CATALYSIS OF LUMINOL CHEMILUMINESCENCE

5 x 10^{-4}M Luminol, 1 x 10^{-2}M Potassium t-butoxide, Dimethyl Sulfoxide Solvent

Metal Catalyst	Adsorbant or Inert Support	I_{max} (10^4 amp)	I_L = Integrated Output (amp-sec)	Average Integrated Output amp-sec)	Remarks
None	Charcoal (acetylene) Photometer(1)	0.4 0.6 0.5	166 21 55	167 ± 12	
None	Charcoal (acetylene) Photometer(2)	0.9 0.7 0.5	169 164 224	168 ± 14	Average of six runs is 177 ± 21
None	Alumina (basic)	1.3	361		
None	Silica	1.0	380		
None	Molecular Sieve (5A)	3.1	606		
None	Teflon	3.0 3.4	631 545	588 ± 43	
Platinum	Teflon	0.5	227		
Platinum	Charcoal	0.4	177		1% Pt by weight; metal ppt. directly on membrane
Platinum	Charcoal	0.5	220		3% Pt by weight; metal ppt. directly on membrane
Platinum	Charcoal	0.63 0.65	214 243	229 ± 15	Pt ppt. on charcoal prior to fabrication of membrane
Palladium	Teflon	0.04	55		
Silver	Teflon	2.2 2.0	576 503	540 ± 37	
Cobalt	Teflon	0.1	173		
Talc[a]	Teflon	1.3 .5	667 144		
Neutral Alumina[a] (active)	Teflon	1.2	253		
Zinc Oxide[a]	Teflon	0.6	387		
Magnesium Oxide[a]	Teflon	2.6	264		

a) On the day this membrane was run, the Teflon standard membrance was found to have an integrated light output of 338 ± 75.

A solution of 5×10^{-5}M sensitizer in the presence and absence of 2×10^{-2}M base was excited with 3000-4000 Å excitation. The results are recorded in Tables 26 and 27. It can be seen in Table 26 that, in general, the intensity of emission is strong both in neutral and alkaline solution. In several cases, the fluorescence color shifts, e.g., thionine eosinate, ANSA, and acridine red. Two sensitizers, BBO and ANSA, were less soluble in alkaline solution than in neutral solution. A smaller number of sensitizers was screened for stability to oxidation in the presence of luminol prior to photometric measurement (Table 27). The color of the solution and the fluorescence emission were the same before and after oxidation except for methylene blue and its zinc double salt.

A representative sample of fluorescent agents from each class of dyes and scintillators was chosen from these Tables. The photometric measurements are summarized in Tables 28 and 29. In all cases, but one, the chemiluminescence emission color did not match the fluorescence color of the sensitizer in alkaline solution. The exception was fluorescein. In some cases, the emissions were the color of luminol (e.g., POPOP) while in other cases, a blending of color from the sensitizer and luminol, (e.g., Eosin Y and Erythrosin). The majority of the sensitizer experiments were conducted with basic alumina membranes since dyes were adsorbed on the surface.

For fluorescein the color of the fluorescence from the membrane on which fluorescein dianion was absorbed was the same as that in solution. However, Eosin Y adsorbed on basic alumina emitted an orange fluorescence, while in the solution, the color was yellow green. Any enhancement of the quantum yield of fluorescence due to adsorption on the membrane would have improved the chemiluminescence efficiency of luminol. The absorption of dyes by alumina indicated that this membrane is active, even though it was slightly discolored during fabrication.

Since the color of the chemiluminescence emission of luminol in the presence of fluorescein dianion corresponds to that of the dye, a sensitivity correction was made for the spectral response of the photomultiplier at the emission wavelength. This was necessary in order to access any enhancement of the quantum yield (Table 28).

Teflon membranes gave the best results in both peak intensity and integrated light output. The lower current reading obtained in the presence of fluorescein was due to the lower sensitivity of the photomultiplier at the fluorescein emission wavelength, and because the fluorescence efficiency of fluorescein is less than one. An approximate value was calculated for the relative current reading expected for emissions from luminol (I_L) and fluorescein (I_F) as detected by the RCA 1P28 photomultiplier (ref. 6). Assuming equal radiant power emitted from the two sources, the ratio I_F/I_L was found to be 0.6 (see Appendix III).

Table 26

PROPERTIES OF ORGANIC FLUORESCENT AGENTS IN NEUTRAL AND ALKALINE DIMETHYSULFOXIDE SOLUTIONS(a)

Sensitizer(b) 5×10^{-5}M	Color of Solution Neutral Solution	Color of Solution 2×10^{-2}M Base	Fluorescence Intensity Neutral Solution	Fluorescence Intensity 2×10^{-2}M t-BuOK	Color of Fluorescence Neutral Solution	Color of Fluorescence 2×10^{-2}M t-BuOK
Fluorescein	---	green	---	strong	---	yellow
Acridine Yellow	yellow-green	maroon	strong	strong	yellow	yellow
Rhodamine 6G	red	blue	strong	---	orange	red
Acridine Orange	yellow-brown	yellow	strong	strong	yellow-green	yellow-green
Acridine Red	violet	light tan	strong	weak	orange	brown
Methylene Blue (Zn Double Salt	blue	yellow-brown	---	strong	---	orange
Erythrosin	purple	red-brown	strong	strong	yellow	yellow-green
Thionine Eosinate	purple	purple	strong	strong	yellow	yellow-green
Decacycline	yellow	yellow	strong	strong	green	green
Pentacene	light purple	poorly soluble	medium	---	yellow-white	---
Rubrene	yellow-orange	weakly soluble yellow	weak	weak	yellow-orange	yellow-orange
α-NOPON	colorless poorly soluble	colorless poorly soluble	strong	strong	blue	blue
α-NPO	colorless	colorless	strong	strong	blue	blue
BBO	colorless	colorless moderately soluble	strong	strong	blue	blue
PBD	colorless	colorless	strong	---	blue	---
POPOP	colorless	colorless	strong	strong	blue	blue
PPO	colorless	colorless	medium	medium	blue	blue
ANSA	colorless	yellow moderately soluble	strong	medium	blue	blue
Chloranil	yellow	yellow	---	---	---	---
Rhodamine B	---	decomposes	strong	---	red	---
Rose Bengal	purple-pink	purple-pink	strong	strong	red-orange	orange-yellow
Euchrysine	yellow-orange	yellow-green	medium	medium	green-yellow	green
Safranine 6B	purple-pink	blue	medium	medium	orange	red
Magdala Red	purple	pale green	medium	weak	orange-yellow	green
Eosin Y	pink	pink	strong	strong	yellow	green-yellow
Safranine O	purple-pink	blue	medium	medium	orange	deep red
Methylene Blue	blue	pale yellow	---	medium	---	orange-red

(a) Fluorescene excited by 3000-4000 A in a 1 cm quartz cell.

(b) Abbreviations:

α-NOPON: p-Bis-[2-(5,1-naphthyloxazoly)]-benzene

α-NPO: 2-(1-Naphthyl)-5-phenyloxazole

BBO: 2,5-Dibiphenyloxazole

PBD: Phenylbiphenyloxadiazole

POPOP: p-Bis-[2-(5-phenyloxazolyl)]-benzene

PPO: 2,5-Diphenyloxazole

ANSA: 4-Amino-1-naphthalenesulfonic Acid

Table 27

Preliminary Results of Chemiluminescence Emission from Luminol in the Presence of Fluorescent Agents(a)

Sensitizer	Color of Solution Prior to Oxidation	Color of Fluorescence of Solution Prior to Oxidation	Color of Chemiluminescence	Color of Solution after Oxidation	Color of Fluorescence of Oxidized Solution
Rose Bengal	pink	orange	pale blue	pink-purple	orange
Methylene Blue Zn Double Salt	yellow	pale green	pale blue	green	green-yellow
Erythrosin	orange-pink	green-yellow	pink-blue	pink	orange-yellow
Euchrysine	yellow-green	green	green-blue	green-yellow	green
Safranine 6B	blue	green	pale blue	blue	green
Magdala Red	green	green	green-blue	green	green
Eosin Y	orange	yellow-green	orange-blue	pink	yellow
Safranine O	deep blue-green	green	pale blue	blue	green
Methylene Blue	yellow-green	green-yellow	pale green blue	green	green
Acridine Yellow	brown-pink	green	pale-blue	yellow-brown	green-yellow

a) 5×10^{-5}M Fluorescer, 5×10^{-4}M Luminol and 10^{-2}M t-BuOK solution in a 1.0 cm quartz absorption cell.

TABLE 28

HETEROGENEOUS CATALYSIS OF LUMINOL IN THE PRESENCE OF FLUORESCEIN

5×10^{-4}M Luminol, 1×10^{-2}M Potassium t-butoxide, Dimethyl Sulfoxide Solvent

Metal	Adsorbant or Inert Support	Concentration of Fluorescein (Molar x 10^3)	I_{max} (10^4 amp)	I_F = Integrated Light Output (amp-sec) X 10^4	Experimental Integrated Light Output Ratio $\frac{I_F}{I_L}$	Corrected [b] Light Output Ratio $\frac{2.1 I_F}{I_L}$	N% [c]
None	Teflon	5.0	1.6 1.8 1.4	313 330 365	0.57 ± 0.06	1.14	14
None	Teflon	0.5	2.7	549	0.93	1.86	86
Silver	Teflon	5.0	1.4	297	0.55	1.10	10
Platinum	Teflon	5.0	0.3	160	0.70	1.40	40
Palla-dium	Teflon	5.0	0.02	48	0.87	2.61	161
None	Alumina, basic	0.5	.8 0.6	230 213	0.64 0.59	1.28 1.18	28 18
	"	5.0	0.5	259	0.71	1.42	42
	"	0.5	1.3	236	0.65	1.30	30
None	Silica	5.0	0.5	166	0.44	0.88	-12
None	Charcoal	5.0 5.0 .5	0.2 0.2 0.5	184 95 148	0.59 0.54 0.84	1.18 1.08 1.68	18 8 68
Platinum	Teflon	—	0.4	177	a		

a) 5×10^{-4}M Rhodamine B; Orange solution became colorless and non-fluorescent upon addition of base.
b) Corrected I_F/I_L value. See Appendix for calculation. Fluorescein fluorescence yield 0.8
c) Percentage increase in light yield due to energy transfer to fluorescein.

Table 29

CATALYZED EMISSION IN THE PRESENCE OF FLUORESCENT AGENTS

Fluorescent Agent	Imax (10^4 amp)	Integrated output (10^4 amp-sec)	Color of chemiluminescence	Color of fluorescence of oxidized solution	Color of oxidized solution	Color of fluorescence of membrane	Remarks
BASIC ALUMINA MEMBRANE (5×10^{-4}M Luminol, 5×10^{-4}M Fluorescer, 10^{-2}M t-BuOK)							
Fluorescein	--	--	--	--	yellow	yellow	Basic solution of sensitizer is yellow
Acridine Orange	1.3	474	--	--	--	yellow	
Rhodamine 6G	1.1	158	blue	yellow-green	tan	--	
Eosin Y	1.20 0.86	431 381	blue, sprinkled with maroon	pale orange or yellow-green	green	orange	
Erythrosin	0.65	--	blue, sprinkled with maroon	pale orange or yellow-green	--	orange	Emission abruptly decreased after 3-1/2 minutes of oxidation
POPOP	1.5	300	blue-green	blue	yellow	none	
Safranine 6B	1.1	204	greenish-blue	blue	purple	none	
Euchrysine	1.6	356	greenish-blue	blue	yellow	none	
Magdala Red	0.86	186	greenish-blue	--	brown	none	
Rose Bengal	0.96	183	blue	--	pink-purple	pink	
Methylene Blue	1.0	262	blue-green	green	purple	none	
Acridine yellow	0.88	320	greenish-blue	--	--	--	
Methylene Blue double zinc salt	(0.94)	(281)	greenish-blue	green	--	none	Membrane leaked
Decacyclene	--	--	blue-green	green	--	yellow-brown	
TEFLON MEMBRANE (5×10^{-5}M Fluorescer, 10^{-2}M t-BuOK, 5×10^{-4}M Luminol)							
Acridine Orange	1.8	536	green	yellow	--	--	
Rhodamine 6G	1.5	410	blue	yellow-green	brown		
Eosin Y	1.1	241	--	--	--		
POPOP	2.0	435	blue	blue	yellow		
Decacyclene	--	--	blue	green	yellow-brown		

If the energy that is detectable as light in the unsensitized chemiluminescence is quantitatively transferred to fluorescein, the expected current decrease would be $\emptyset$ (I_F/I_L) or 0.5 for the sensitized chemiluminescence.

The relative integrated light output in the presence and absence of sensitizer, R, can be corrected for the photomultiplier spectral response and fluorescence efficiency of fluorescein by multiplying R by [$\emptyset(I_F/I_L)$], $\emptyset$ = 0.80.

At the fluorescein concentration of 5×10^{-5}M, there is a gain of approximately 86 per cent in light yield over that of unsensitized luminol. A similar observation has been reported for a hydrogen peroxide oxidation system (ref. 34). A greater enhancement of the efficiency may be possible at an optimum sensitizer concentration. At 5×10^{-4}M, the increase in emission is only 14 per cent, presumably because of self-absorption. Changing the fluorescein concentration from 10^{-6}M to 10^{-4}M showed that the chemiluminescence efficiency was greater at the higher concentration.

The reasons for the difference in behavior between Teflon and alumina (Table 28) involve (1) changes in fluorescein concentration by adsorption during the course of the oxidation, (2) possible deactivation of the alumina after adsorption of the sensitizer, and (3) changes in the reflectivity of the membranes. There are insufficient data available to distinguish among these explanations.

Both palladium and charcoal exhibited large corrected I_F/I_L values. For a palladium membrane, the percentage increase in the light yield was higher than that observed for a homogeneous (Teflon membrane) reaction. The systems may be further improved by determining the optimum concentration of sensitizer.

F. CHEMILUMINESCENCE OF TETRAKIS DIMETHYLAMINOETHYLENE (TMAE) IN THE PRESENCE OF SOLID SUBSTRATES

Since pure TMAE leaks through a Teflon membrane, a solvent system compatible with the membrane was sought. The TMAE solvent, n-decane, also goes through the membrane. The results of this solvent screening program is recorded in Table 30. Two solvents that were compatible with the Teflon membrane when it contained traces of water, permeated it when was dried over alumina. In wet diglyme and ethyl ether, TMAE generates heat, but produces a very weak chemiluminescence (Table 31). TMAE was either insoluble or nonchemiluminescent in other solvents screened.

G. CHEMILUMINESCENCE FROM THE OXALYL CHLORIDE-HYDROGEN PEROXIDE-DIPHENYL ANTHRACENE SYSTEM

The chemiluminescence from oxalyl chloride-hydrogen peroxide reaction has been found to proceed with high efficiency (ref. 35). The use of membranes that decompose hydrogen peroxide, e.g.,

Table 30

COMPATABILITY OF SOLVENTS WITH MEMBRANE

Solvents Which Leak Through Teflon Membranes

p-Xylene	Acetone
Chloroform	Tetrahydrofuran
Dichloromethane	Ethyl ether (dry)
Tetrachloroethane	Diglyme (dry)
Dioxane	Monoglyme
Triethyl amine	n-Hexyl ether
n-Hexylamine	Mesetyl oxide
H.P.T.	n-Decane

Solvents Which Do Not Leak Through Teflon Membranes

Dimethylformamide	Nitromethane
Diethyl benzoate	Triglyme
Acetonitrile	Methyl cellosolve
Diethyl malonate	
Ethyl ether (wet)	
Diglyme (wet)	

Table 31

SOLUBILITY AND REACTIVITY OF TETRAKIS DIMETHYL AMINO ETHYLENE IN SOLVENTS COMPATIBLE WITH TEFLON MEMBRANE

Solvent	Observations
Dimethylformamide	Insoluble
Diethyl malonate	Colloidal suspension, no chemiluminescence
Acetonitrile	Insoluble
Diethyl benzoate	Soluble, but no chemiluminescence
Diethylphthlate, dry	Solution turns red, no heat generated, no chemiluminescence
No Solvent	TMAE wets membrane
Methyl Cellosolve	Insoluble
Wet Ethyl Ether	Solution turns red, heat liberated

platinum and alumina, was considered as a way to increase light emission. The water content in the solvent, diethylphthalate (DEP), determined by the Karl Fisher technique, was 4.4×10^{-2}M, (ref. 36). To prepare a stable stock solution of oxalyl chloride in DEP, the solvent was distilled over sodium hydride under reduced pressure. Hydrogen peroxide was added to DEP by shaking 300ml of the solvent with 200ml of 30% aqueous hydrogen peroxide overnight.

The concentration of H_2O_2 in DEP was determined by the sodium iodine sodium thiosulfate method (ref. 37). Hydrogen peroxide was found to 0.38M in DEP, and was stable to decomposition for the length of time required to investigate the influence of solid substrates on the oxalyl chloride system.

To prevent metal-catalyzed decomposition of H_2O_2, a thin Teflon tubing was slipped over the end of the stainless steel syringe needle. The Teflon tip was inserted in the liquid so that a mixing of the reagents would be more efficient. The upper chamber of the cell was exposed to the atmosphere. The total light yield is reported in Table 32. The total, graphically integrated, light emission was highly reproducible. Two membranes, neutral alumina and silver, gave light yield increases of about 15% relative to Teflon. Platinum vigorously decomposed H_2O_2 with diminished light production.

H. CHEMILUMINESCENCE OF LOPHINE IN THE PRESENCE OF SOLID SUBSTRATES

The base-catalyzed autoxidation of lophine in alcoholic alkaline solution is known to be weakly chemiluminescent (ref. 33). The dimethyl sulfoxide-potassium t-butoxide system was used in our study in an attempt to improve the chemiluminescence efficiency of the oxidation. The membranes tested were Teflon, basic alumina, charcoal, and silica gel. A solution of 5×10^{-3}M lophine and 0.1M t-BuOK yielded a light intensity barely detectable above the dark current.

Table 32

CHEMILUMINESCENCE OF H_2O_2-OXALYL CHLORIDE-DPA SYSTEM*

Membrane	Integrated Light Yield amp sec x 10^4
Teflon	6.4
	6.4
	6.4
Carbon	4.2
Basic alumina	5.8
Neutral alumina	7.9
Silica Gel	3.1
Talc	6.0
Zinc Oxide	5.5
Silver	7.6
Magnesium Oxide	6.6

*0.19M H_2O_2, 3.3×10^{-2}M H_2O, 1×10^{-3}M Diphenylanthracene, 2.42×10^{-3} M Oxalyl Chloride

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APPENDIX I

ORGANIC SYNTHESIS

I. SUBSTITUTED ORTHO-AMINOALDEHYDES AND KETONES

A. o-ACETAMIDOBENZALDEHYDE (AAB)

This compound, mp 68.5-70°C (lit. mp 70-71°C), was prepared from o-aminobenzaldehyde and acetic anhydride by the method of Camps (ref. 27).

B. o-FORMAMIDOACETOPHENONE (FAP)

This compound, mp 74-76°C [lit. mp 79°C (ref. 25)], was prepared by the general formylation procedure developed by C. W. Huffman (ref. 26).

C. o-ACETAMIDOACETOPHENONE (AAP)

o-Acetamidoacetophenone, mp 72-74°C (lit. mp 74-75°C) was prepared in 80% yield by the method of Leonard and Boyd (ref. 31).

II. 5-SUBSTITUTED SKATOLE DERIVATIVES

A. GENERAL PROCEDURE

A general method was developed for the preparation of 5-substituted skatoles from the corresponding indole-3-acetic acids. The method involves thermal decarboxylation and distillation at 200°C/0.1 mm in a sublimation apparatus.

1. 5-Methylskatole [mp 74-74.5°C, lit. (ref. 24) mp 74-74.5°C].
2. 5-Benzyloxyskatole [mp 118-119°C, lit. (ref. 23) mp 118°C], picrate [mp 161-161.5°C, lit. (ref. 23) mp 164°C].
3. 5-Methoxyskatole [mp 64.5-66°C, lit. (ref. 22) mp 66°C], pictrate [mp 148-149°C, lit. (ref. 22) mp 151-152°C].

III. INDOLE PEROXIDES

A. 11-HYDROPEROXYTETRAHYDROCARBAZOLENINE

This compound, mp 114°C viol. dec. [lit. mp (rate dependent) 124-129°C viol dec.], was prepared by the method of Beer (ref. 21).

B. 2,3-DIMETHYL-3-INDOLENINYLHYDROPEROXIDE

Five attempts to prepare this hydroperoxide by the literature method (ref. 15) were unsuccessful.

A procedure utilizing platinum metal as an oxidation catalyst (ref. 20) also failed. All attempts to produce this material gave unreacted starting material and polymeric gums.

IV. 2,3-DIMETHYLINDOLE-5-CARBOXYLIC ACID

This indole was prepared in 30% overall yield (three steps) according to the procedure of Verkade and Lieste (ref. 28).

A. 2-(p-CARBETHOXYPHENYL)AMINO-3-BUTANONE (I)

Alkylation of ethyl p-aminobenzoate with 3-bromo-2-butanone in aqueous alcohol with sodium bicarbonate as an acid scavenger provided I as a tan solid (46%) after charcoaling and recrystallization from diethyl ether-petroleum ether (bp 30-60°C); mp 72.5-73.5°C.

B. ETHYL 2,3-DIMETHYLINDOLE-5-CARBOXYLATE (II)

Reaction of I with the hydrochloride salt of ethyl p-aminobenzoate (prepared by treatment of the amino ester with anhydrous hydrogen chloride in absolute ethanol-diethyl ether; mp 206-208°C) yielded II as a light tan solid (70%) with mp 109-111°C. Recrystallization from diethyl ether-petroleum ether (bp 30-60°C) raised the mp to 114-115.5°C. No impurities were detected by VPC and NMR analysis.

C. 2,3-DIMETHYLINDOLE-5-CARBOXYLIC ACID

Saponification of II with alcoholic potassium hydroxide, followed by acidification, provided the acid as a tan solid in 93% yield with mp 237-239°C. Charcoaling and recrystallization did not substantially change the mp (mp 238-239.5°C).

V. 3-ETHYLINDOLE

This compound was prepared in 66% yield by the lithium aluminum hydride reduction of 3-acetylindole according to the procedure of Leete and Marion (ref. 29). The crude product was purified by distillation (bp 83-84°C/13-14 mm); the distillate solidified in the receiver to an off-white solid [mp 33-34°C; reported (ref. 30) 42°C, (ref. 30) 33-35°C]. The infrared spectrum was consistent with the structure.

APPENDIX II

INSTRUMENTATION AND EXPERIMENTAL PROCEDURES

I. PHOTOMETER SYSTEM FOR STATIC CHEMILUMINESCENCE EXPERIMENTS

The chemiluminescence light emission intensity and decay curves were measured by an RCA IP28 photomultiplier and displaced on a Varian chart recorder. The design of the apparatus is schematically represented in Figure 29. The sensitivity of the photomultiplier was checked periodically against a blue tritium phosphor (ref. 46). The photocurrent was adjusted to 5×10^{-6} amp by regulating the high voltage power supply to the photomultiplier. In this way, it was possible to compare runs obtained on different days.

A fritted glass gas dispersor was inserted through the rubber stopper above the test tube. The oxygen employed was General Dynamics high purity grade. The gas was further purified by passage through a molecular sieve 5A and Dry Ice-actone trap.

II. PHOTOMETER SYSTEM FOR DYNAMIC CHEMILUMINESCENCE EXPERIMENTS (FLOW SYSTEM)

A steady state flow apparatus, with a water-jacket was constructed (Figure 30). A constant temperature could be maintained by closed loop water recirculation. The major objectives of the flow system were to measure emission spectra at low light levels, and to permit convenient evaluation of optimum reagents and solvent ratios.

III. SPECTROMETER AND CALIBRATION

The corrected spectral curves and light intensities reported for chemiluminescence and fluorescence emission were measured with Model 1700 Spex Czerny-Turner scanning spectrometer. The spectrometer was equipped with a 1200-groove/mm grating blazed at 5000Å and an E.M.I. 9558Q photomultiplier. A double optical

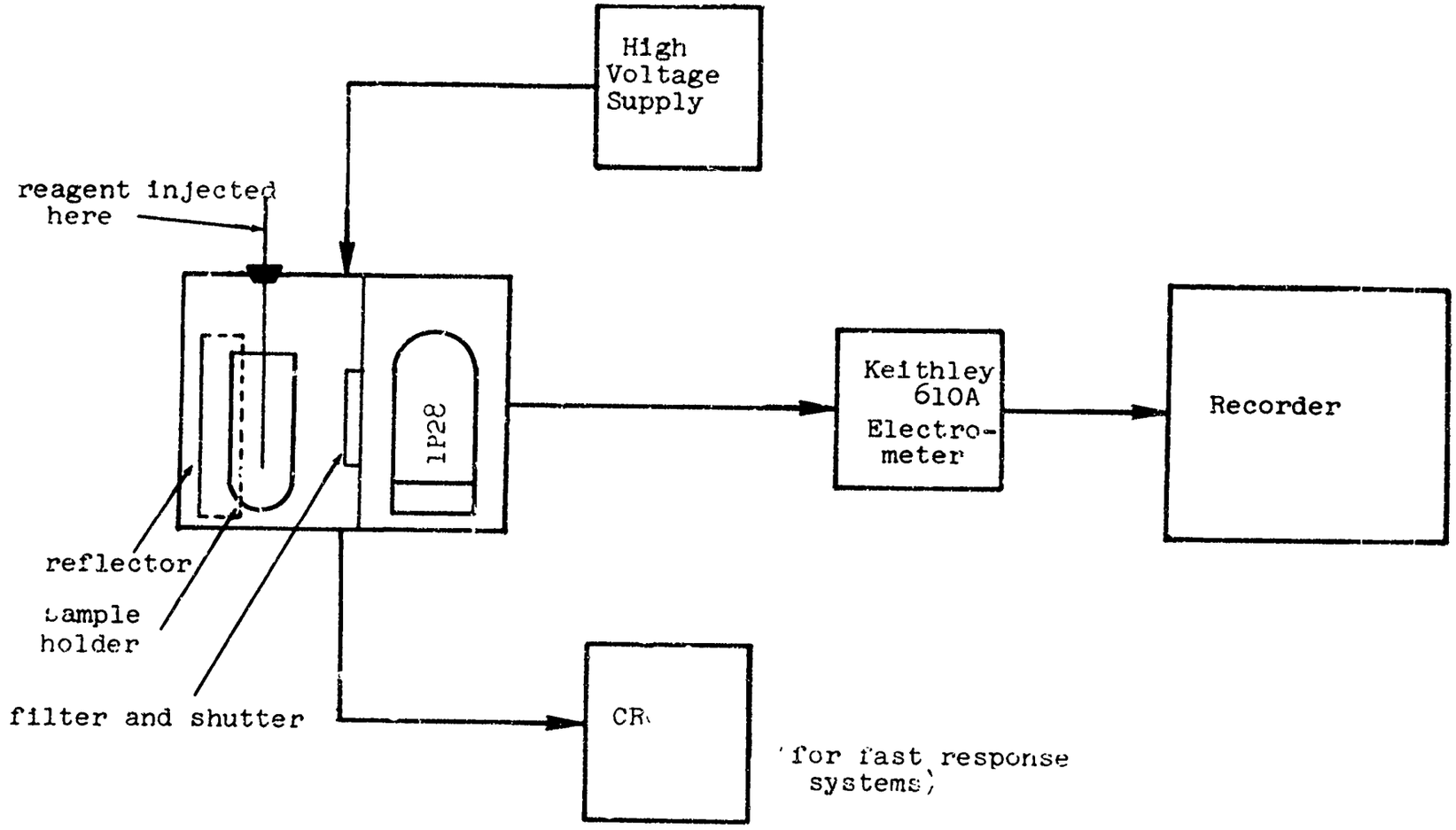

Figure 29. Static Photometer System

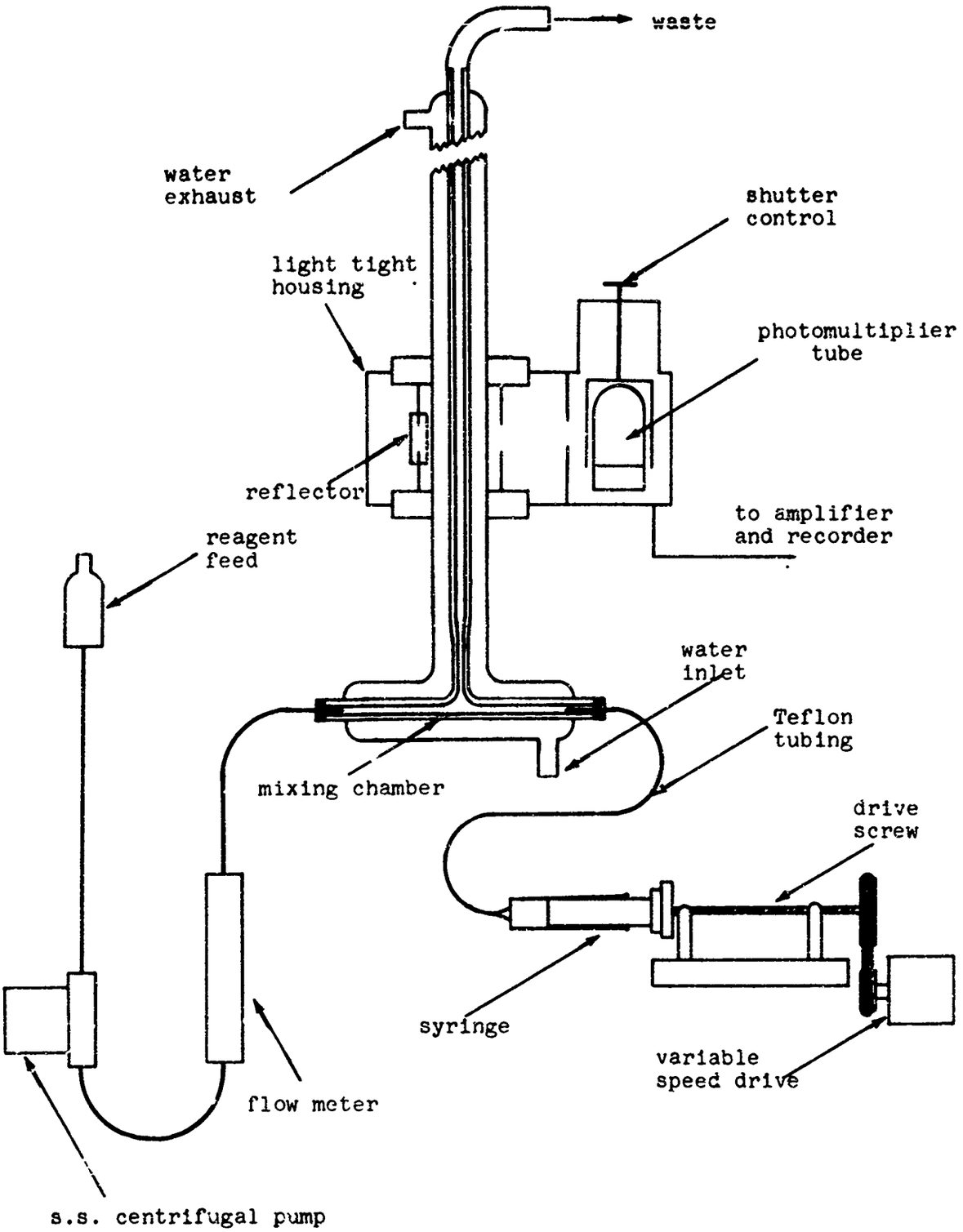

Figure 30. Flow Photometer

bench was employed that permitted determination of fluorescence, chemiluminescence, and absorption spectra on a single sample (Figure 31).

The determination of the absolute fluorescence spectra, quantum yields, and brightness of chemiluminescent reactions requires calibration of the spectral sensitivity of the spectrometer. A relative calibration of the spectral sensitivity of our spectrometer in the near ultraviolet and visible spectrum by comparison to fluorescence standards was performed.

The fluorescence standards were selected from the literature (ref. 48, 49) to cover the spectral range between 320 mμ and 660 mμ. A low-pressure mercury lamp was used as the excitation source, oriented at a 90° angle relative to the spectrometer.

The conditions under which the fluorescence standards were used is given below:

(1) 5.0×10^{-4}M naphthalene in ethanol; M.C.B., recrystallized from ethanol, mp 79-80°C; spectral range 324 to 370 mμ (ref. 48); 254 mμ excitation.

(2) 4.3×10^{-6}M anthracene in ethanol; Eastman Kodak blue-violet fluorescent grade, used without further purification, spectral range 370 to 400 mμ (ref. 48); 254 mμ excitation.

(3) 1×10^{-4}M quinine sulfate in 1N sulfuric acid; M.C.B., mp 229°C; (the sulfate was used without further purification) spectral range 400 to 500 mμ (ref. 49); 3130 and 3330 Å excitation*.

(4) 1×10^{-4}M N,N-dimethyl-m-nitroaniline in 30% by volume fluorescent grade benzene and 70% by volume of M.C.B. spectrograde heptane**, spectral range 500 to 660 mμ (ref. 49); 3130 and 3330 Å excitation.

The emitters were chosen so that the wavelength of each spectrum is overlapped by the adjacent spectra, permitting a continuous relative spectral sensitivity calibration. The

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* The literature mp is 235.2°C. The lower melting point is attributed to the presence of water of recrystallization; mp of quinine sulfate $\cdot 2H_2O$ is 205°C.

** M.C.B. spectrograde hexane contained a trace amount of fluorescent material and was not used. Lippert reports the spectrum in this solvent, but the spectrum will be similar in heptane.

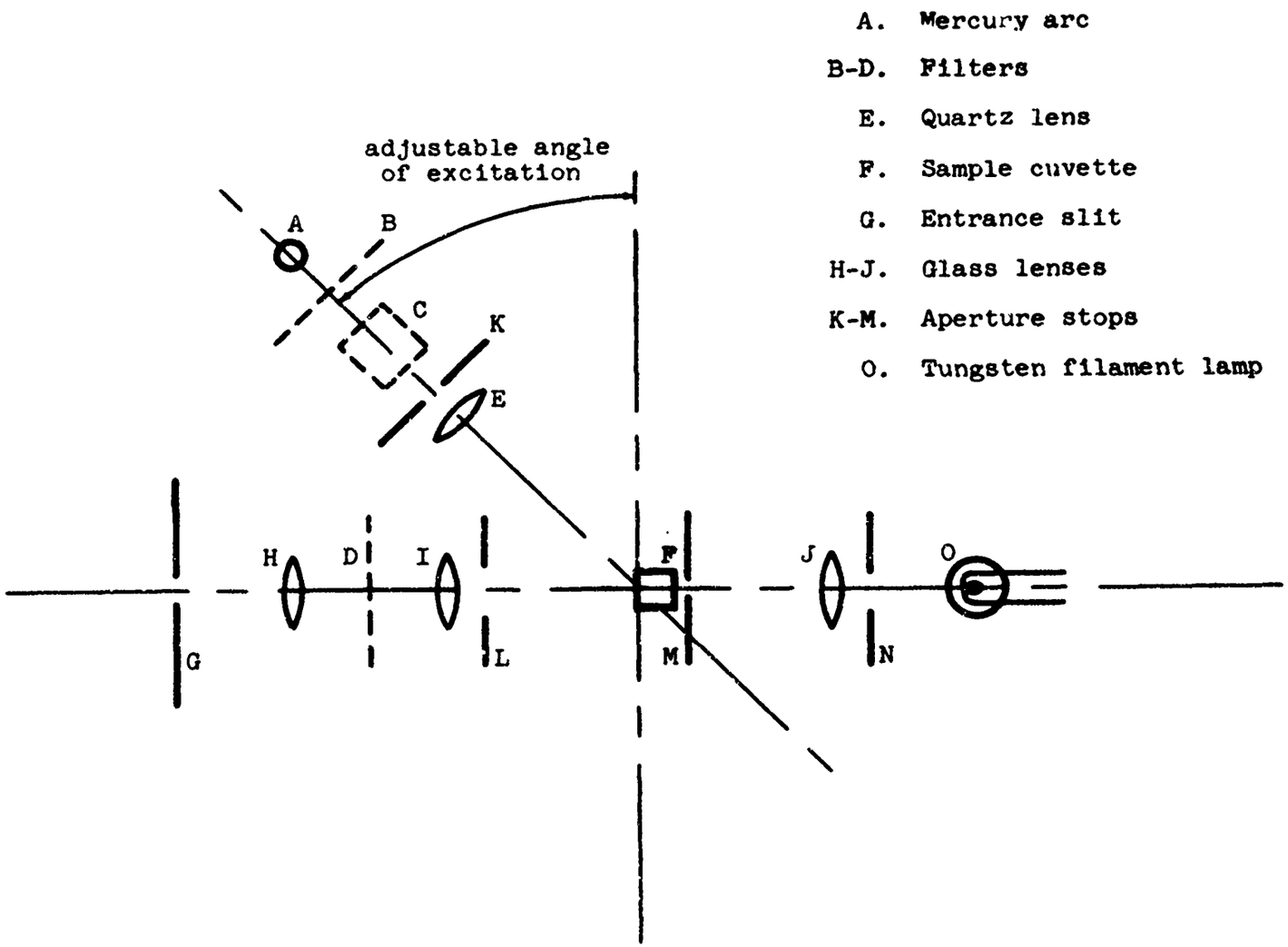


Figure 31. Schematic Diagram of Optical Bench for Fluorescence, Chemiluminescence and Absorption Measurements

sensitivity of the instrument is obtained by taking the ratio of the observed photocurrent, measured in microamperes, at a given wavelength and bandwidth, to the true relative spectral distribution of the emitter in units of photons/unit frequency interval. To obtain the relative sensitivity, the ratio at 376 mμ was defined as unity. The sensitivities at the wavelengths where the anthracene intensity crosses naphthalene and quinine sulfate were calculated, and the relative sensitivity continued as a smooth function through the wavelengths of these emitters.

The relative sensitivity function obtained is shown in Figure 32. The curve is characterized by two maxima, at 376 and at 441 mμ. The spectral range covered by each compound is designated in the legend.

The wavelength of the exciting source was chosen as to be near the maxiumum of the absorption band. This selection should reduce the effect of traces of fluorescent impurities. The concentrations were chosen to minimize the effects of self absorption. The gross features of the sensitivity function resembles the reported quantum efficiency curve of the photomultiplier in the blue and that reported by Parker for a similar system (ref. 50).

IV. HETEROGENEOUS CATALYSIS APPARATUS

The cells designed for the quantitative measurement of the effect of heterogeneous catalysis is shown in Figure 27 and Figure 28 in the text. Each cell consists of two chambers, A and B, isolated by a gas-permeable membrane. The membrane support consists of a Teflon-coated, fine-mesh, stainless steel screen. The catalytic solid is affixed to one side of the membrane with a Teflon binder.

In Cell 1, before the solution was introduced into Chamber B, both chambers were purged with nitrogen. After a sufficient time elapsed for de-aeration, nitrogen-purged solutions of the reactant and base were injected above the membrane. The chamber containing the oxidizable solution was continually flushed with nitrogen during the course of the oxidation to prevent backup of water vapor and oxygen through the gas exhaust arm. Oxygen was then introduced into Chamber A at any desired pressure. The spectrally integrated light output was measured photometrically with a IP28 photomultiplier.

The vertical orientation of the photomultiplier with respect to the solution facilitated variation of the depth of the solution from zero to any convenient value. In this way, self-absorption by the solution was minimized.

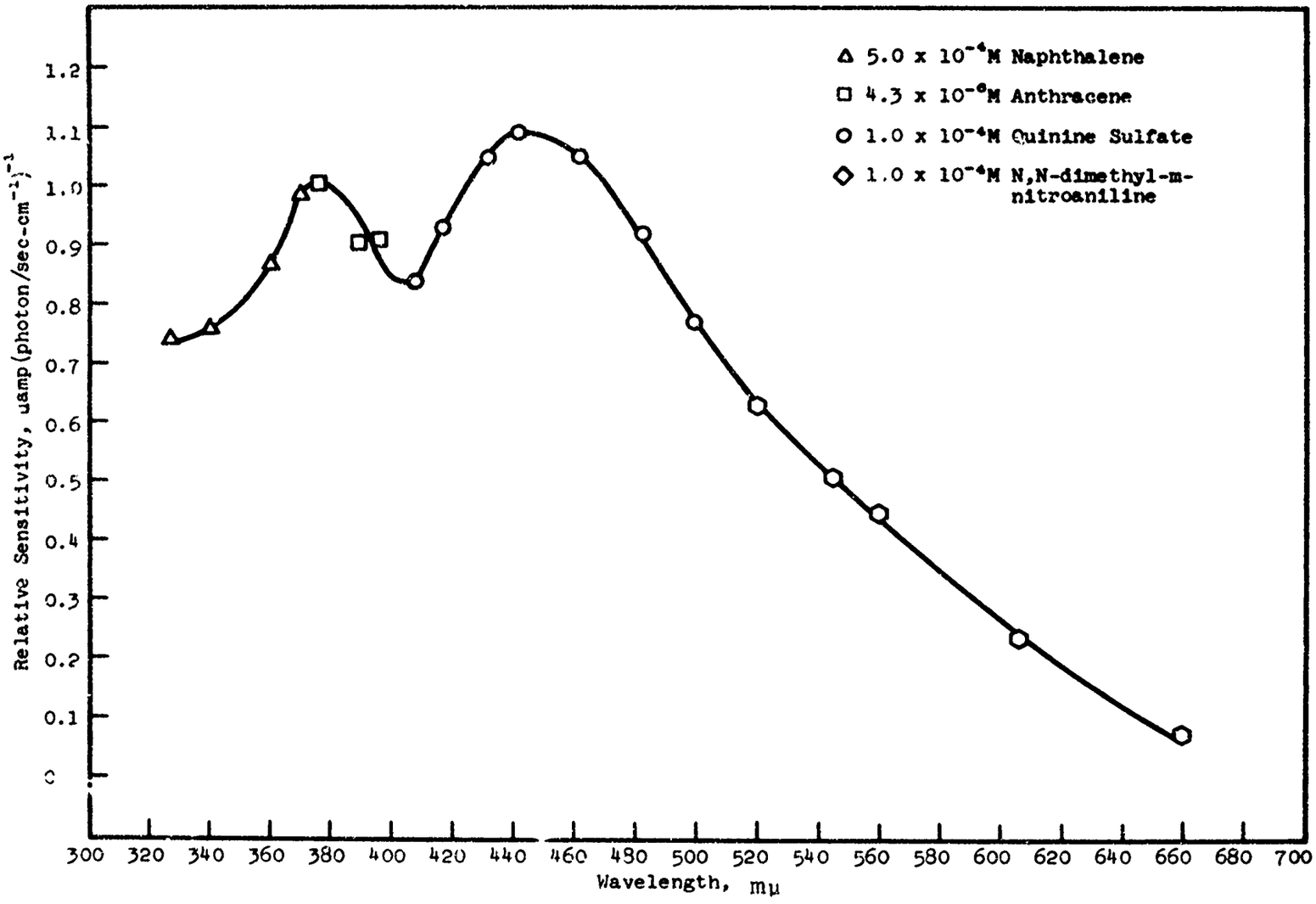


Figure 32. Relative Sensitivity of Spectrometer vs Wavelength

After several experiments with luminol chemiluminescence, it was discovered that the nitrogen purging agitated the liquid and produced non-uniform emission of light. In Cell 2, the design is basically the same as in Cell 1 except that the upper chamber is isolated from the atmosphere.

Liquid solutions were freshly prepared in a nitrogen atmosphere and injected above the membrane through the right side-arm while nitrogen is flowing through the left side-arm. After the solutions were introduced into the chamber, a stopcock was attached, and the interconnecting tubing flushed with nitrogen. Both stopcocks were then closed, and dry oxygen was introduced into the lower chamber at a rate of less than 50 ml/min (measured at 25°C and 1 atmosphere pressure). The open construction of the lower chamber insured that the pressure of oxygen at the membrane surface was one atmosphere.

APPENDIX III

CALCULATION OF THE RELATIVE PHOTOCURRENTS PRODUCED BY LUMINOL AND FLUORESCEIN EMISSION

To determine the relative current yield produced by luminol with and without fluorescein sensitization, the spectral sensitivity of the photomultiplier and power distribution of the emitters should be known. These factors are related in the following equation:

$$I = G\int_0^\infty W(\lambda)\, R(\lambda)\, d\lambda, \qquad (1)$$

where I = photocurrent, amp
G = geometric factor
$W(\lambda)$ = radiant power, watts per unit wavelength
$R(\lambda)$ = receiver response, amp per watt

Since the power distribution is unknown, the above equation can be modified to incorporate the total energy from the source, W_o, and the normalized spectral distribution $N(\lambda)$.

Since $$W(\lambda) = \frac{N(\lambda)}{\int_0^\infty N(\lambda)d\lambda} W_o \qquad (2)$$

equation (1) becomes

$$I = \frac{G\int_0^\infty N(\lambda)\, R(\lambda) W_o\, d\lambda}{\int_0^\infty N(\lambda)\, d\lambda} = \frac{GW_o \int_0^\infty N(\lambda)\, R(\lambda)\, d\lambda}{\int_0^\infty N(\lambda)\, d\lambda}$$

The relative current expected from two light sources, fluorescein (F) and luminol (L) is:

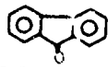
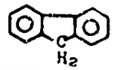
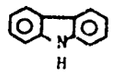
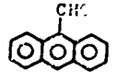
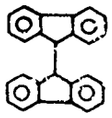
$$\frac{I_F}{I_L} = \frac{W_{o_F} \int_0^\infty N_F(\lambda)\, R(\lambda)\, d\lambda \Big/ \int_0^\infty N_F(\lambda)\, d\lambda}{W_{o_L} \int_0^\infty N_L(\lambda)\, R(\lambda)\, d\lambda \Big/ \int_0^\infty N_L(\lambda)\, d\lambda}$$

The 1P28 photomultiplier spectral response curve is that characteristic of an S-5 response. For the situation of equal radiant power emitted from both sources W_{oF} is equal to W_{oL}. Graphical determination of the above integrals for the fluorescein-luminol case gave a value of I_F/I_L of 0.6.

APPENDIX IV

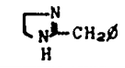
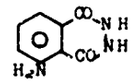
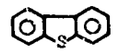
SCREENING DATA

Table 1

Compound and Structure	Run	Sensitization* or Reaction Condition	Conc.** t-BuOK, molar	Initial Pulse,†,‡ amp	Peak‡,§,†† O_2 Current, amp	Time to O_2 Peak	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
Dimethyl Sulfoxide (DMSO) $(CH_3)_2SO$	a			-	9×10^{-11}	15 min	light yellow		≈4×10^{-11}amp before O_2 add (Spectro). (3)1.2×10^{-11} before O_2 add (Reagent). Note ten-fold increase of luminescence with DPA.
	b			(3)10^{-9}	(3)2.9×10^{-11}	29 min			
	c	DPA		(3)5×10^{-10}	(5)8.7×10^{-11} (3)3×10^{-10}	4 sec 31 min			
Dimethyl formamide (DMF) $(CH_3)_2NCHO$	a		0.2	-	10^{-9}	50 min	precipitate		solvent decomposition with precipitation
	b	0.5M in DMSO			10^{-9}	7 min	pink	yellow	
9-Fluorenone 	a				2.5×10^{-9}	4.5 min	red-brn		Note luminescence and increase in DMF with precipitate.
	b	2.5×10^{-3}M			10^{-9}	4.5 min			
	c	In DMF			1.8×10^{-8}	1 min	pink	yellow	
Benzophenone Ø CO Ø					9×10^{-11}	13 min	yellow		No increase in luminescence over solvent system.
Fluorene 	a				8×10^{-9}	9 min	red-brn	color deepens	Fairly bright luminescence.
	b	In DMF			10^{-8}	2 min	orange		With precipitate.
Carbazole 					9×10^{-10}	3 min	yellow		
9-Anthracenealdehyde 					5×10^{-9}	4 min	brown solution		
Dianisalacetone $(CH_3O-C_6H_4-CH=CH)_2CO$	a				1.5×10^{-10}	5 min	brown solution		weak luminescence
	b		0.05		1.5×10^{-10}	9 min	solution red-brn		
	c	TPB	0.05		2.7×10^{-10}	3.5 min			sensitized emission
	d	TP	0.05		1.5×10^{-10}	5 min			
Diphenylfulvene $Ø_2C=C_5H_4$	a		0.05	10^{-8}	-	-			No O_2 effect.
	b				5×10^{-10}	30 sec	dark solution		
	c	TPB			5×10^{-10}	30 sec	dark solution		No sensitization.
9,9'-Bifluorenyl 					1.2×10^{-9}	4 min	red solution		Secondary buildup.
					1.5×10^{-9}	.6 min			

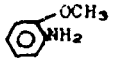
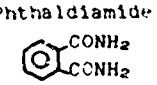
NOTES: See end of Table.

Table 1 (continued)

Compound and Structure	Run	Sensitization* or Reaction Condition	Conc.** t-BuOK, molar	Initial Pulse,†,‡ amp	Peak‡,§,†† O_2 Current, amp	Time to O_2 Peak	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
Acetone CH_3COCH_3					6×10^{-10}	2 min	yellow	dark brn	
Benzoin	a				$(5\pm1)10^{-8}$	3.5±0.5min	dark red-brn	yellow	Dark green ⟶ blue intermediate.
H ØCOHCOØ	b	DPA			6×10^{-8}	4 min			Peak luminescence in blue ⟶ yellow transition (visible luminescence).
	c				10^{-7}	50 sec			O_2 flow rate ≈0.7 cc/sec.
	d		0.016		10^{-7}	2 min	dark green	yellow	
	e		0.0035		2×10^{-11}	40 sec			Addition of same conc. base ⟶ pulse $>10^{-7}$ amp.
2-Benzylimidazoline 				$(3\pm1)10^{-7}$				yellow	Addition of O_2 or N_2 decreased luminescence (visible luminescence).
Benzil	a			5×10^{-9}			yellow		No sensitization - no effect with O_2.
	b	DPA		5×10^{-9}					
ØCOCOOØ	c	TP		5×10^{-9}					
	d	TPB		5×10^{-9}					
Benzaldehyde	a			10^{-8}			yellow		Not distilled - no O_2 effect.
	b			10^{-8}	10^{-9}	15 sec			Freshly distilled.
Ø CHO	c			3×10^{-9}					Distilled and aged 24 hr (also d,e).
	d	DPA		3×10^{-9}					No O_2 effect.
	e	TPB		10^{-8}					Initial pulse sensitized.
Luminol 	a				5×10^{-6}		dark brown		Note sensitivity to base concentration.
	b		0.008	9×10^{-6}			yellow		
	c		0.015	6.4×10^{-4}	5.8×10^{-4}	30 sec	yellow		Decay to 10^{-4} before O_2 addition.
Thiourea H_2NCSNH_2	a			6.4×10^{-9}	4.5×10^{-10}	2 sec	yellow	orange	Water and H_2O_2 injection effect minor.
	b	DPA		7.6×10^{-9}	4×10^{-10}	2 sec			
Anisonitrile	a			3×10^{-9}	1.5×10^{-10}	10 sec	yellow	orange	
	b	DPA		2.3×10^{-9}	1.4×10^{-10}	5 sec			
CH_3O ⬡ CN					2.4×10^{-10}	28 min			Note long slow rise.
	c	TP		6.5×10^{-9}	8×10^{-11}	5 sec			
	d	TPB		9×10^{-9}	7×10^{-10}	25 sec			Weak sensitization.
Dibenzothiophene 	a			3.6×10^{-9}	4×10^{-10}	3 min	colorless	red-brn	O_2 peak is three-fold greater than background.
	b			$(3)2.7\times10^{-8}$	$(3)1.1\times10^{-10}$	30 sec			
	c	DPA		$(3)5\times10^{-9}$	$(3)5\times10^{-11}$	10 sec			Time to peak variable.
	d	TPB		$(3)5\times10^{-8}$	$(3)10^{-10}$	50 sec			No sensitization.
	e	TPB + rubrene		$(3)10^{-9}$	-	-			

NOTES: See end of Table.

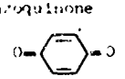
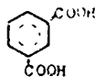
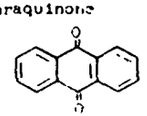
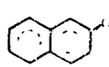
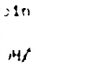
Table 1 (continued)

Compound and Structure	Run	Sensitization* or Reaction Condition	Conc.** t-BuOK, molar	Initial Pulse,†,‡ amp	Peak‡,§,†† O_2 Current, amp	Time to O_2 Peak	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
Benzalazine Ø CH=N-N=CH Ø	a			3.7×10^{-10}	8×10^{-11}	15 min			Oscillation in output during O_2 addition - see text. Average is DMSO base.
	b	TPB		5.3×10^{-10}	2.6×10^{-10}	11 min			Oscillations and average amplified by TPB
Acetonitrile CH_3CN	a			8×10^{-10}	6×10^{-11}	13 min			O_2 peak is DMSO base.
	b	TPB		10^{-9}	6×10^{-11}	5 min			
	c	TP		3×10^{-9}	6×10^{-11}	-			Compares to sensitization in pure DMSO.
	d	DPA		10^{-9}	9×10^{-10}	21 min			
Azobenzene Ø N=N Ø	a			(3)6.5x10	(3)1.5x10	5 min	orange	dark red-brn	Steady state signal independent of O_2. Note inhibition of DMSO luminescence.
	b		0.025	$(3)10^{-10}$	zero	-	orange	lt brn	
o-Anisidine 	a			$(3)10^{-9}$	$(3)5\times10^{-11}$	20 sec	colorless	deep red-brn	Luminescence and sensitization effects both small.
	b		0.05	$(3)10^{-9}$	$(3)2.4\times10^{-11}$	2 min			
	c	TPB	0.05	$(3)3\times10^{-9}$	$(3)7.2\times10^{-11}$	15 sec			
	d	DPA	0.05	$(3)10^{-9}$	$(3)5.3\times10^{-11}$	40 sec			
	e	TP	0.05	$(3)2.6\times10^{-9}$	$(3)4.4\times10^{-11}$	5 sec			
	f	TP + rubrene	0.05	$(3)2.4\times10^{-9}$	$(3)3.8\times10^{-11}$	40 sec			
	g	rubrene + fluorescein	0.05	$(3)1.7\times10^{-9}$	$(3)6.2\times10^{-11}$	50 sec			
Phthaldiamide 	a			$(3)1.2\times10^{-9}$	$(3)6\times10^{-11}$	1 min	clear	turbid	
	b	DPA		$(3)1.2\times10^{-9}$	-	-			No increase with O_2.
	c	TPB		(3)2x10-9	$(3)7.5\times10^{-11}$	20 sec			Marginal sensitization.
U-dimethylhydrazine $(CH_3)_2NNH_2$	a			$>(3)3\times10^{-9}$	$(3)4.8\times10^{-11}$	15 sec	lt yellow	dk yellow	Luminescence weak.
	b	TPB		$(3)1.4\times10^{-9}$	$(3)4.5\times10^{-11}$	14 sec			No sensitization.

NOTES TO TABLE 2:

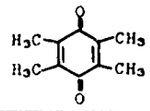
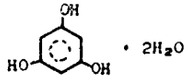
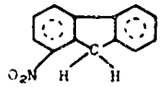
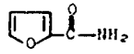
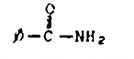
- Sensitizers at 10^{-4} mole/liter. Reactant 5×10^{-3}M; solvent DMSO unless otherwise noted.
  TP = p-terphenyl
  TPB = 1,1,4,4-tetraphenylbutadiene
  DPA = 9,10-diphenylanthracene
  POPOP = 2,2'-p-phenylene-bis-(5-phenyloxazole)
- ** Concentration 0.1 mole/liter unless otherwise noted.
- † Initial or mixing pulse values are given where they exceed the current observed with addition of O_2.
- ‡ The parenthetical factors given in some current values are required for equivalent sensitivity of different photometers. Thus the current value $(3)2.9\times10^{-11}$ is to be read as 8.7×10^{-11}.
- § At standard flow rate of 0.35 cc/sec, unless otherwise noted.
- †† Photomultiplier current maintained $\leq10^{-8}$ amp by attenuation filters where required.

Table 2

Compound and Structure	Run	Sensitizer*	Initial Pulse ratio	Peak O_2 Current ratio	Time to O_2 Peak	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
p-Benzoquinone 	a		2.0×10^{-3}	10^{-4}	4.5 min		dk brn	
	b	DPA	2.6×10^{-3}	10^{-4}	3 min		dk brn	
Isophthalic acid 	a	DPA	2×10^{-2}	4×10^{-4}	20 sec	orange-yellow		
	b		$>2\times10^{-2}$	4×10^{-4}	5 sec	orange-yellow		
	c	TIB	4×10^{-2}	6×10^{-4}	2 sec	yellow		
	d	TP	3×10^{-2}	6×10^{-4}	25 sec	yellow		
Anthraquinone 	a		2×10^{-2}	3×10^{-4}	80 sec		rd org	
	b	DPA	2×10^{-2}	7×10^{-4}	30 sec		orange	
	c	TPB	3×10^{-2}	10^{-3}	35 sec		orange	
	d	TP	3×10^{-2}	7×10^{-4}	45 sec		orange	
2 Naphthol 	a		2×10^{-2}	5×10^{-4}	5 sec			two oxidation peaks with O_2
				7×10^{-4}	25 min			secondary peak
	b	DPA	1.5×10^{-2}	2×10^{-4}	5 sec			secondary peak
				7×10^{-4}	10 min			no effect with sensitizer
Benzoin 	a		10^{-3}	$(1.2\pm0.2)\times10^{-1}$	62±? sec		yellow	solvent DMF
	b	DPA	10^{-3}	2×10^{-4}	57 sec		yellow	solvent DMF
	c		5×10^{-4}	4×10^{-2}	4 min 50 sec		yellow	solvent DMSO
	d	DPA	7×10^{-4}	1×10^{-1}	2 min 20 sec		yellow	solvent DMSO
	e		10^{-3}	0.5	14 sec			recrystallized, fritted dispersor

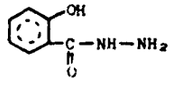
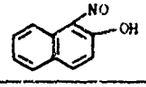
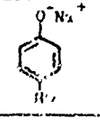
* See end of Table.

Table 2 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse ratio	Peak O_2† Current ratio	Time to O_2 Peak‡	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
Duroquinone 	a		5×10^{-4}	1.7×10^{-3}	6M, 20S	dk rd	dk rd brn	
	b	DPA	3×10^{-4}	1.7×10^{-3}	7 min	dk rd	dk rd brn	No effect with sensitizers
Phloroglucinol 	a		1×10^{-3}	3×10^{-4}	15M,40S	clear yellow	cloudy yellow	With fluorescent surface
	b	DPA	1×10^{-3}	2×10^{-4}	15 min			
	c	TPB	1×10^{-3}	6×10^{-4}	3M,15S			
2-Nitrofluorene 	a		10^{-4}	10^{-4}	16 min	deep green	reddish violet	lower than blank
2-Furamide 	a		$>2\times10^{-3}$	4×10^{-4}	6 sec	clear yellow	clear yellow	
	b	DPA	4×10^{-3}	3×10^{-4}	11.5min			
Benzamide 	a		$>5\times10^{-3}$	6×10^{-4}	15 sec		yellow	
	b	TP	$>2\times10^{-2}$	2×10^{-3}	25 sec		yellow	
	c	DPA	10^{-2}	10^{-3}	35 sec		yellow	
	d	TPP	2×10^{-2}	1.0×10^{-3}	15 sec		yellow	

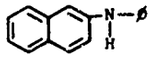
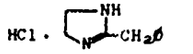
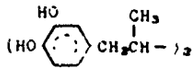
[illegible] end of Table

Table 2 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse ratio	Peak O_2† Current ratio	Time to O_2 Peak‡	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
Salicylhydrazide 	a		$>>2\times10^{-3}$	5×10^{-3}	30 sec	yellow	greenish yellow	
	b	TP	1×10^{-2}	2×10^{-3}	5 min	yellow	greenish yellow	
	c	DPA	$>2\times10^{-2}$	5×10^{-3}	4 min	yellow	greenish yellow	O_2 signal persists for ≈4 min.
	d	TPB	4×10^{-2}	4×10^{-3}	3M,15S	yellow	greenish yellow	O_2 signal persists for ≈4 min.
Oxamide	a		$>2\times10^{-3}$	2×10^{-4}	30 sec		lt yellow	precipitate
	b	DPA	$\approx2\times10^{-3}$	1.5×10^{-4}	20 sec		lt yellow	no precipitate
	c	TP	6×10^{-3}	3×10^{-4}	5 sec		orange-yellow	
Hydroquinone	a		1.5×10^{-4}	6×10^{-4}	5 min		brownish-orange	
	b		1×10^{-3}	6×10^{-4}	2M,40S		brownish-orange	O_2 flowing when base was injected.
	c	TP	$>6\times10^{-4}$	5×10^{-4}	5 min			
1-Nitroso-2-naphthol 	a		$(9.0\pm0.8)\times10^{-2}$	$(3\pm1)\times10^{-4}$	≈5 sec		deep green	Visible yellow emission with base injection.
	b		$(1.1\pm0.0)\times10^{-2}$	$(1.4\pm0.6)\times10^{-4}$	<5 sec		brownish-red	"Practical Grade" Recrystallized.
p-Nitrosophenol Sodium Salt 	a		5×10^{-4}	2×10^{-4}	20 min		green	

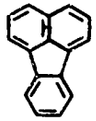
† See end of Table.

Table 2 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse ratio	Peak O_2† Current ratio	Time to O_2 Peak†	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
N-phenyl-2-naphthylamine 	a		1.5×10^{-3}	1×10^{-4}	20 sec	orange	dk brn	
2-Benzylimidazoline·HCl 	a b		10^{-2} 6×10^{-3}	10^{-3}	6 min			Aldrich Columbia
Pyrocatechol			10^{-3}	2×10^{-4}	20 min			
Benzalazine $\phi-N=N-\phi$			6×10^{-4}	6×10^{-5}	16 min	yellow	rd-brn	No oscillations
Nordihydroguaiaretic acid 	a b	TP	6×10^{-3} 5×10^{-3}	2×10^{-4} 2×10^{-4}	8 min 9 min		orange orange	No effect with sensitization.
Triphenylamine ϕ_3N	a b c	DPA TP	2×10^{-3} 5×10^{-3} 2.5×10^{-3}	3×10^{-4} 3×10^{-4} 2×10^{-4}	16 min 21 min 18 min	yellow yellow yellow	orange orange orange	

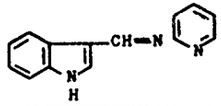
NOTE: See end of Table

Table 2 (continued)

Compound and Structure	Run	Sensitizer[a]	Initial[†] Pulse ratio	Peak O_2[†] Current ratio	Time to O_2 Peak[‡]	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
Indene	a		10^{-2}	1.5×10^{-3}	20 sec	yellow	blackish brown	
	b	DPA	10^{-2}	1×10^{-3}	10 sec	yellow	blackish brown	No effect with sensitization.
	c	TP	10^{-2}	1.5×10^{-3}	30 sec	yellow	blackish brown	No effect with sensitization.
	d	TPB	1.3×10^{-2}	1.5×10^{-3}	50 sec	yellow	blackish brown	No effect with sensitization.
Fluoranthene 	a		1.5×10^{-3}	4×10^{-2}	50 sec	yellow	blue-purple	
	b	DPA	1.5×10^{-3}	4×10^{-2}	35 sec	yellow	blue-purple	No apparent effect with sensitization.
	c	TP	1.5×10^{-3}	3×10^{-2}	40 sec	yellow	blue-purple	No apparent effect with sensitization.
	d	TPB	1.5×10^{-3}	3×10^{-2}	40 sec	yellow	blue-purple	No apparent effect with sensitization.
	e	Rubrene	1×10^{-3}	2.5×10^{-2}	2 min	yellow	reddish	No apparent effect with sensitization.
Fluoranthene (recrystallized)	a		1.5×10^{-3}	6×10^{-2}	50 sec		dp purple	
	b		6×10^{-3}	1.3×10^{-1}	10 sec			Fritted dispersor.
	c	DPA	2×10^{-2}	1.7×10^{-2}	50 sec			
	d	Rubrene	1.5×10^{-3}	2.5×10^{-2}	90 sec			
	e	TP	1.7×10^{-3}	6×10^{-2}	50 sec			
	f	TPB	1.5×10^{-3}	4×10^{-2}	30 sec			
Triphenylmethane ϕ_3CH	a		10^{-3}	2.5×10^{-4}	19 min			
Biuret $H_2NCONHCONH_2$	a		2×10^{-3}	3×10^{-4}	20 min			

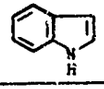
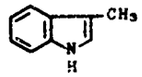
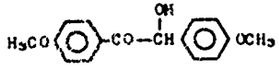
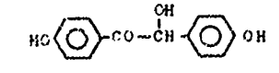
NOTES: See end of Table.

Table 2 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse ratio	Peak O_2† Current ratio	Time to O_2 Peak†	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
3(N-2-Pyridyl-formimidoyl) indoles 	a		$\approx 10^{-2}$	4×10^{-3}	20 min			O_2 flow rate 0.08 SCFM instead of 0.04 SCFM.
	b	Rubrene	8×10^{-3}	3×10^{-3}	3 min			O_2 flow rate 0.08 SCFM instead of 0.04 SCFM.
	c	TP	7×10^{-3}	3×10^{-3}	14 min			O_2 flow rate 0.08 SCFM instead of 0.04 SCFM.
	d	TPB	1.3×10^{-2}	3.5×10^{-3}	3 min			
Hydrazinedihydrochloride $H_2N{-}NH_2\cdot 2HCl$	a		2×10^{-2}	10^{-3}	15 sec			Second peak ≈ same height as first peak at t ≈ 20 min.
	b	DPA	10^{-2}	6×10^{-4}	5 sec			
	c	Rubrene	6×10^{-3}	3×10^{-4}	4 sec			
	d	TP	6×10^{-3}	3×10^{-4}	5 sec			
Benzhydrol ϕ_2COH H	a		2×10^{-3}	2×10^{-4}	20 min			Peak signal increased spontaneously: O_2 had little immediate effect.
	b	Rubrene	1.2×10^{-2}	8×10^{-4}	20 min			
	c	DPA	3×10^{-3}	2.5×10^{-4}	16 min			
Cinnamaldehyde $\phi{-}CH{=}CH{-}CHO$	a		1.5×10^{-1}	4×10^{-4}	11 min		reddish	
	b	DPA	1.5×10^{-1}	5×10^{-4}	10 min		reddish	
	c	TP	2×10^{-1}	7×10^{-4}	5 sec			
	d	TPB	3.4×10^{-1}	7×10^{-4}	5 sec		reddish	
	e	Rubrene	1.5×10^{-1}	7×10^{-4}	15 sec		red	
4-Hydroxy-4-methyl-2-pentanone $CH_3{-}\underset{\displaystyle CH_3}{\overset{\displaystyle |}{C}}OH{-}CH_2COCH_3$			10^{-3}	2×10^{-3}	6 min			
1,3-Dihydroxy-2-propanone $HOCH_2COCH_2OH$			10^{-4}	1.5×10^{-4}	12 min			No enhanced chemiluminescence.

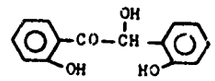
NOTES: See end of Table.

Table 2 (continued)

Compound and Structure	Run	Sensitizer[a]	Initial[f] Pulse ratio	Peak O_2[f] Current ratio	Time to O_2 Peak[g]	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
Indole 			1.6	0.30	66 min			
Skatole 	a b c	Fluorescein		60 140 80				Fritted dispersor for all. t-BuOK, 0.067 M, for all. Skatole 5×10^{-3} M Skatole 10^{-2} M Skatole 10^{-2} M (See text for other results.)
Diphenylamine ϕ_2NH	a b	DPA	4×10^{-3}	7×10^{-3} 6×10^{-3}	2.5 sec 15 sec		dk green	Fritted dispersor.
Anisoin 	a b c	DPA TP	10^{-3} 2×10^{-3} 10^{-3}	$(6.6\pm0.8)\times10^{-2}$ 0.15 6.6×10^{-2}	20±4 S 23 sec 24 sec	same color sequence as benzoin		Fritted gas dispersor.
Tetraphenylhydrazine $\phi_2N-N\phi_2$	a b	DPA	2×10^{-3}	3×10^{-3} 7.4×10^{-3}	3.5 min 17 sec			Fritted gas dispersor
4,4'-Dihydroxybenzoin 	a b c d	DPA + RUB TP DPA	2.4×10^{-3} 4×10^{-3} 6×10^{-3} 7×10^{-3}	2.4×10^{-3} 2.6×10^{-2} 4×10^{-2} 4×10^{-2}	30 sec 40 sec 60 sec 60 sec			Fritted gas dispersor. Does not form dark solution in base.

NOTES: See end of Table.

Table 2 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse ratio	Peak O_2† Current ratio	Time to O_2 Peak‡	Appearance Before O_2 Add.	Appearance After O_2 Add.	Comments
2,2'-Dihydroxybenzoin 	a		1.8×10^{-2}	4.5×10^{-3}	18 sec			Fritted dispersor. Color sequence similar to benzoin.
	b	DPA	2×10^{-2}	10^{-2}	15 sec			

NOTES: * Abbreviations

DPA = 9,10-Diphenylanthracene
TP = p-Terphenyl
TPB = Tetraphenylbutadiene
RUB = Rubrene

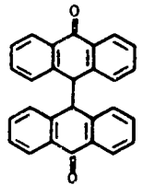
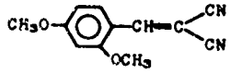
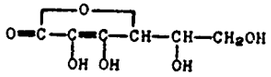
Reaction Conditions

All compounds 5×10^{-3} M in 0.1 M t-BuOK in DMSO; Sensitizers 10^{-4} M unless otherwise noted.

† Ratio of photometer current to photometer current produced by standard source.

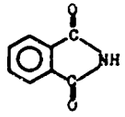
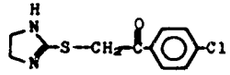
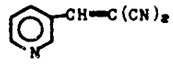
‡ S = second, M = minute

Table 3

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
10,10'-Bianthrone 	a		>2 x 10^{-2}	1.4 x 10^{-3}	15 s	green	red	2.5 x 10^{-3}M (conc. of Bianthrone) $t_{1/2}$ - 35 s
	b		4 x 10^{-2}	5 x 10^{-3}	20 s	green	red	
	c	DPA	1 x 10^{-2}	9 x 10^{-3}	20 s	green	red	
2,4-Dimethoxybenzilidene malononitrile 	a		1.5 x 10^{-3}	2 x 10^{-3}	10 s	yellow	clear	Second peak at $t_{1/2}$ = 200 s I/I_0 = 2.5 x 10^{-3}
	b	DPA	2.4 x 10^{-3}	3.6 x 10^{-3}	20 s	yellow	clear	
L-Ascorbic acid 	a		6 x 10^{-4}	3.6 x 10^{-3}	15 s	yellow	clear	Second peak at $t_{1/2}$ = 95 s I/I_0 = 2.4 x 10^{-3}
	b	DPA	2.4 x 10^{-3}	4.4 x 10^{-3}	15 s	yellow	clear	Second peak at $t_{1/2}$ = 3 m I/I_0 = 2 x 10^{-3}
2,3,4,5-Tetraphenyl pyrrole	a		2.1 x 10^{-3}	2.4 x 10^{-3}	50 s			$t_{1/2}$ = 360 s
	b	DPA	1.2 x 10^{-2}	3.6 x 10^{-3}	40 s			$t_{1/2}$ = 270 s
	c	Rubrene	5.4 x 10^{-3}	1.8 x 10^{-3}	30 s			$t_{1/2}$ = 510 s

Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
1-Phenyl-1,3-butanedione	a		1.4×10^{-2}	4×10^{-3}	1 m			
	b		1.3×10^{-2}	4×10^{-3}	72 s			
	c	DPA	8×10^{-3}	4×10^{-3}	72 s			
	d	Rubrene	1.5×10^{-2}	2.4×10^{-3}	90 s			
Triphenylphosphine	a		6.6×10^{-2}	4.2×10^{-5}	75 s			
	b	DPA	7.4×10^{-3}	3.6×10^{-3}	65 s			
	c	Rubrene	5×10^{-3}	2×10^{-3}	10 s			
Phthalimide 	a		5×10^{-3}	4×10^{-3}	1 m			
	b	DPA	6×10^{-3}	4×10^{-3}	50 s			
2-(p-Chlorophenylacylthio)-2-imidazoline 	a		2×10^{-3}	3.6×10^{-3}	200 s			
	b	DPA	2.4×10^{-3}	3.6×10^{-3}	3 m			
3-Pyridylmethylene malononitrile 	a		6.4×10^{-3}	3.6×10^{-2}	4 m			
	b	DPA	5×10^{-3}	4.5×10^{-2}	3 m			

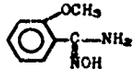
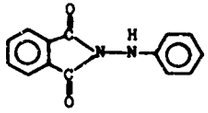
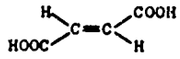
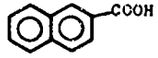
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
Acrylonitrile $H_2C{=}C{=}CH{-}CN$	a b c	DPA Rubrene	1×10^{-3} 5×10^{-3} 1.2×10^{-2}	1.2×10^{-2} 1.7×10^{-2} 1.9×10^{-2}	28 s 28 s 25 s	clear clear lt orange	red red dk red	$t_{1/2}$ = 75 s $t_{1/2}$ = 170 s $t_{1/2}$ = 220 s
Methylvinylketone $H_2C{=}CH{-}C(=O){-}CH_3$	a b c	DPA Rubrene	2.4×10^{-2} 1.8×10^{-2} 4.0×10^{-2}	8.2×10^{-2} 6.6×10^{-2} 8×10^{-2}	10 s 7 s 7 s	clear clear lt orange	dk red dk red dk red	$t_{1/2}$ = 21 s $t_{1/2}$ = 21 s $t_{1/2}$ = 21 s
Anisic acid hydrazide $CH_3O{-}C_6H_4{-}C(=O){-}NH{-}NH_2$	a b	DPA	2×10^{-4} 6×10^{-4}	5×10^{-4} 6×10^{-4}	7 m 5 m			
Cyanoacetohydrazide $NC{-}CH_2{-}C(=O){-}NH{-}NH_2$	a b	DPA	2×10^{-4} 4×10^{-4}	8×10^{-4} 6×10^{-4}	5 s 5 s	clear clear	red red	
2,5-Diphenyl-1,3,4-oxadiazole $C_2N_2O(C_6H_5)_2$			6×10^{-4}	3.6×10^{-4}	40 s			
N-Furfurylphthalamic acid $C_{13}H_{11}NO_5$	a b	DPA	2.4×10^{-3} 2.2×10^{-3}	1.6×10^{-3} 2×10^{-3}	10 s 10 s			

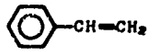
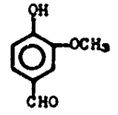
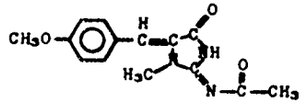
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer[a]	Initial[f] Pulse Ratio	Peak O_2[f] Current Ratio	Time to[g] O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
o-Anisamidoxime 	a b	DPA	5×10^{-4} 5×10^{-4}	6×10^{-4} 4×10^{-4}	5 s 5 s			
N-Anilinophthalimide 	a b	DPA	4×10^{-4} 5×10^{-4}	1.2×10^{-3} 6×10^{-4}	6 m 1 m			
Fumaric acid 	a b	Rubrene	5×10^{-3} 3.3×10^{-3}	2.4×10^{-3} 7×10^{-4}	6 m 5 s			
Isoquinoline	a b	DPA	1.6×10^{-3} 1.7×10^{-3}	1.7×10^{-3} 2×10^{-3}	10 s 10 s			
2-Naphthoic acid 	a b	DPA	2.4×10^{-3} 4×10^{-3}	1.5×10^{-3} 2.4×10^{-3}	5 m 5 s	clear clear	red red	Second peak at $t_{1/2}$ = 8 m $I/I_0 = 2.4 \times 10^{-3}$

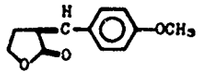
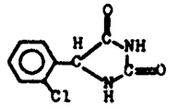
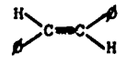
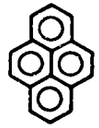
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer[e]	Initial[f] Pulse Ratio	Peak O_2[f] Current Ratio	Time to[g] O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
Acrolein (stabilized with hydroquinone 100 ppm) 	a b	DPA	8×10^{-2} 8.6×10^{-2}	7.0×10^{-2} 7.2×10^{-2}	5 s 5 s	clear clear	lt red lt red	$t_{1/2}$ = 11 s $t_{1/2}$ = 10 s
Styrene (stabilized with t-butylpyrocatechol) 	a b	DPA	9×10^{-3} 1.8×10^{-3}	5.6×10^{-3} 9×10^{-3}	13 m 19 m			$t_{1/2}$ = 9 m $t_{1/2}$ = 12 m
Vanillin 	a b	DPA	1×10^{-3} 9.6×10^{-4}	1.4×10^{-3} 2×10^{-3}	45 s 50 s			
N-Methyl-2-pyrrolidone 	a b	DPA	6×10^{-4} 1×10^{-3}	6×10^{-3} 6×10^{-3}	150 s 150 s			
1-Methyl-2-acetylimido-5-(4-methoxy-benzilidene)-4-imidazoline 	a		8.4×10^{-4}	1.2×10^{-3}	210 s			

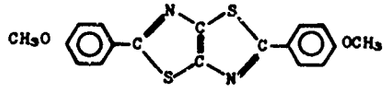
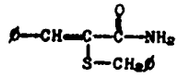
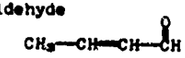
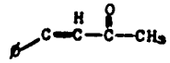
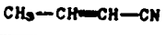
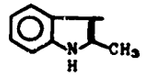
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
α(2-Hydroxyethyl)-p-methoxycinnamic acid - γ-lactone 	a b	 DPA	9.6×10^{-4} 3×10^{-3}	2.2×10^{-3} 1.8×10^{-3}	7 s 4 m			
5-(o-Chlorophenyl)hydantoin 			2×10^{-3}	2.2×10^{-3}	3 m			
Trans-stilbene 	a b	 DPA	1×10^{-3} 1.3×10^{-3}	5×10^{-3} 5.2×10^{-3}	3 m 3 m			
Pyrene 	a b	 Rubrene	4×10^{-3} 4.4×10^{-3}	4×10^{-3} 1.6×10^{-3}	4 m 5 m			
Pyrazine	a b	 DPA	6×10^{-4} 9×10^{-4}	6.4×10^{-3} 3×10^{-3}	3 m 4 m			

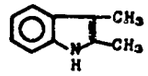
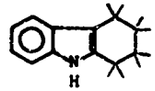
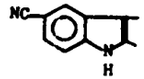
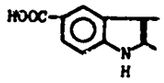
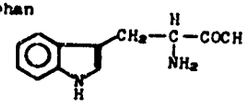
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
2,5-Bis-(4-methoxyphenyl)-thiazolo [5,4-d]thiazole 			1.16×10^{-2}	1.4×10^{-2}	2 m			
α-Benzylthiocinnamide 			4×10^{-3}	1.2×10^{-2}	30 s			
Crotonaldehyde 			2.6×10^{-2}	2.7×10^{-2}	4 s	clear	dk red	$t_{1/2}$ = 6 s
Trans-4-phenyl-3-buten-2-one 	a b	DPA	8×10^{-3} 1.6×10^{-2}	8×10^{-3} 9×10^{-3}	14 s 7 s	clear clear	dk red dk red	$t_{1/2}$ = 17 s $t_{1/2}$ = 20 s
Crotonitrile 			1.0×10^{-2}	1.4×10^{-2}	40 s	clear	dk red	$t_{1/2}$ = 45 s
2-Methylindole 			1.8×10^{-2}	6.0×10^{-2}	30 s			$t_{1/2}$ = 55 s

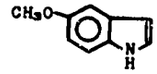
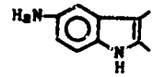
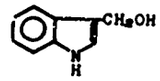
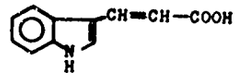
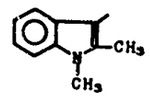
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
2,3-Dimethylindole 			20	60	20 s			$t_{1/2}$ = 60 s
1,2,3,4-Tetrahydrocarbazole 			2.1×10^{-2}	2.6×10^{-2}	20 s			$t_{1/2}$ = 50 s
5-Cyanoindole 			2.6×10^{-2}	2.6×10^{-2}	2 m			$t_{1/2}$ = 9 h
Indole-5-carboxylic acid 			0.2	1.1	3 m			$t_{1/2}$ = 1 h
D-Tryptophan 			8.0×10^{-2}	0.86	90 s			$t_{1/2}$ = 8.5 m

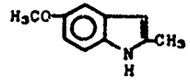
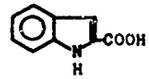
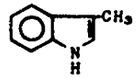
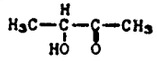
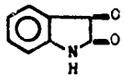
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
5-Methoxyindole 	a b	DPA	1.1×10^{-2} 1.7×10^{-2}	1.3×10^{-2} 2.6×10^{-2}	2 m 1 m			$t_{1/2}$ = 120 s
5-Aminoindole 	a b	DPA	4×10^{-2} 2×10^{-3}	1.1×10^{-2} 1.4×10^{-2}	6 s 7 s			$t_{1/2}$ = 13 s $t_{1/2}$ = 13 s
Indole-3-carbinol 	a b	DPA	9×10^{-2} 9×10^{-2}	8.6×10^{-2} 6×10^{-2}	21 s 26 s			$t_{1/2}$ = 9 m $t_{1/2}$ = 10 m
Indole-3-acrylic acid 	a b	TP	3×10^{-3} 1.3×10^{-2}	1.1×10^{-2} 1.2×10^{-2}	6 m 330 s			$t_{1/2}$ = 16 m $t_{1/2}$ = 20 m
1,2-Dimethylindole 	a b c	TPB Rubrene	2×10^{-3} 2×10^{-3} 3×10^{-3}	1.2×10^{-2} 1×10^{-2} 4×10^{-3}	4 m 150 s 310 s			$t_{1/2}$ = 4.5 m $t_{1/2}$ = 5 m $t_{1/2}$ = 7 m

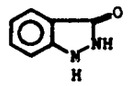
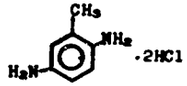
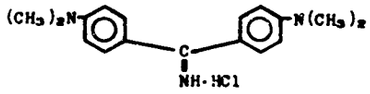
Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
5-Methoxy-2-methylindole 	a b	DPA	7×10^{-3} 8×10^{-3}	1×10^{-2} 8.8×10^{-2}	3 m 3 m			$t_{1/2}$ = 6 m $t_{1/2}$ = 7 m
Indole-2-carboxylic acid 			6×10^{-3}	5×10^{-3}	25 s			$t_{1/2}$ = 5 m
Skatole (3-Methylindole) 			10	2.4×10^{2}	60 s			K^+tBuO^- conc. 0.067 M, solvent DMF, skatole conc. 5×10^{-3}, $t_{1/2}$ = 210 s
3-Hydroxy-2-butanone (Acetoin) 	a b	DPA	2.2×10^{-3} 2.4×10^{-3}	1.8×10^{-3} 3.3×10^{-3}	4 m 40 s	dk brown dk brown	clear clear	
Isatin 	a b	Rubrene	1.2×10^{-2} 1.4×10^{-2}	$\approx 1 \times 10^{-2}$ 1.1×10^{-2}	10 s 10 s	clear	orange	

Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
3-Indazolinone 	a		6×10^{-2}	1.6×10^{-3}	2 m			
	b	DPA	1.4×10^{-1}	8×10^{-3}	3 m			
	c	Rubrene	1.2×10^{-1}	3.3×10^{-3}	45 s			
2,5-Toluenediaminedihydrochloride 	a		4×10^{-4}	2.2×10^{-4}	30 s		deep red	
	b	Rubrene	1.4×10^{-1}	2.2×10^{-4}	5 s		deep red	
Auramine O 	a		8×10^{-3}	7.6×10^{-3}	20 s		brownish-yellow	
	b	Rubrene	8×10^{-3}	5×10^{-3}	25 s		brownish-orange	
	c	DPA	5×10^{-3}	2.8×10^{-3}	270 s		brownish-yellow	
Acriflavinhydrochloride	a		5×10^{-3}	3.1×10^{-4}	390 s			
	b	DPA	4.4×10^{-3}	7×10^{-4}	5 s			
	c	Rubrene	1×10^{-3}	2.5×10^{-4}	210 s			
3,6-Diaminoacridinehemisulfate-hemihydrate	a		2×10^{-3}	5×10^{-4}	15 s		deep red	
	b	Rubrene	$1.x 10^{-3}$	2×10^{-4}	10 s		deep red	
	c	DPA	5×10^{-3}	2.4×10^{-4}	10 s		deep red	

Notes are at the end of this table.

Table 3 (continued)

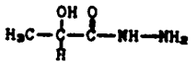
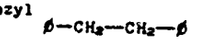
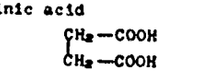
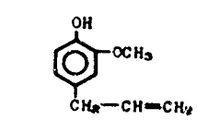
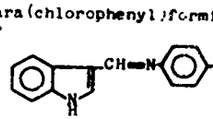
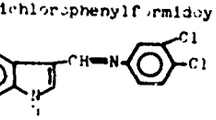
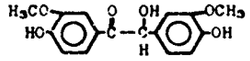
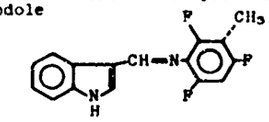
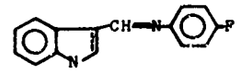
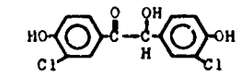
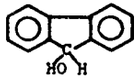
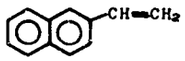
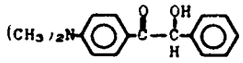
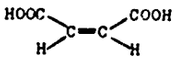
Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
Lactic acid hydrazide 	a b c	DPA Rubrene	2.4×10^{-3} 7×10^{-3} 1.1×10^{-2}	2×10^{-3} 1.3×10^{-3} 1.6×10^{-3}	5 s 150 s 5 s			
Bibenzyl 	a b	Rubrene	9×10^{-3} 4.2×10^{-3}	1.8×10^{-3} 1.2×10^{-3}	5 s 5 s			
Succinic acid 	a b	DPA	5×10^{-3} 5×10^{-3}	1.5×10^{-3} 1.4×10^{-3}	5 s 5 s			
Acridine	a b c	DPA Rubrene	0.4 0.4 0.3	5.0 5.0 2.0	15 s 30 s 5 s		red red red	
Eugenol 	a b c	DPA Rubrene	2×10^{-2} 1.8×10^{-2} 2.6×10^{-2}	1.4×10^{-2} 1.3×10^{-2} 4×10^{-3}	45 s 3 m 1 m			
3-N-Para(chlorophenyl)formidoyl-indole 	a b	DPA	1.7×10^{-2} 4.4×10^{-2}	1.3×10^{-2} 1.3×10^{-2}	5 s 5 s			$t_{1/2} = 5$ s
N-3,4-Dichlorophenylformidoylindole 	a b c	DPA Rubrene	1.3×10^{-2} 1.4×10^{-2} 5×10^{-3}	3.7×10^{-3} 2.8×10^{-3} 4×10^{-3}	2 m 210 s 4 m			

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
4,4'-Dihydroxy-3,3'-dimethoxybenzoin 	a		6×10^{-2}	3.4×10^{-3}	25 s	colorless solution		
	b	Rubrene	7×10^{-3}	4×10^{-3}	5 s	colorless solution		
3-N-Metatrifluoromethylformidoyl-indole 	a		8×10^{-3}	7×10^{-3}	7 m			
	b	DPA	5×10^{-3}	2×10^{-3}	4 m			
	c	Rubrene	5×10^{-3}	3×10^{-3}	5 m			
3-N-Parafluorophenylformidoylindole 	a		6×10^{-3}	5×10^{-3}	4 m			
	b	Rubrene	3×10^{-3}	1.5×10^{-3}	270 s			
3,3'-Dichloro-4,4'-dihydroxybenzoin 	a		3×10^{-2}	5×10^{-3}	5 s	colorless solution		
	b	DPA	2.7×10^{-3}	5×10^{-3}	3 s			
	c	Rubrene	7×10^{-3}	2.4×10^{-3}	5 s			
Acrylic acid stabilized with p-methoxyphenol; $H_2C{=}CH{-}COOH$	a		6×10^{-4}	2.5×10^{-3}	150 s			Did not turn dark $t_{1/2} = 5$ m
	b	DPA	2×10^{-4}	3.2×10^{-3}	210 s			Did not turn dark $t_{1/2} = 3.5$ m

Notes are at the end of this table.

Table 3 (continued)

Compound and Structure	Run	Sensitizer*	Initial† Pulse Ratio	Peak O_2† Current Ratio	Time to‡ O_2 Peak	Appearance Before O_2	Appearance After O_2	Comments
9-Hydroxyfluorene 	a b	DPA	2×10^{-3} 2×10^{-3}	9.2×10^{-2} 6.4×10^{-2}	35 s 20 s			$t_{1/2}$ = 30 s $t_{1/2}$ = 40 s
2-Vinylnaphthalene 	a b	DPA	1.2×10^{-2} 1.2×10^{-2}	2.6×10^{-3} 3.6×10^{-3}	5 m 150 s			$t_{1/2}$ = 5 m $t_{1/2}$ = 7 m
4-Dimethylaminobenzoin 			2×10^{-3}	4×10^{-1}	23 s	deep purple	clear	Same color sequence as benzoin $t_{1/2}$ = 7 s
Maleic acid 	a b	DPA	2×10^{-3} 1×10^{-3}	2.4×10^{-3} 9×10^{-4}	5 m 4 m			

NOTES: *Abbreviations
DPA - 9,10-Diphenylanthracene
TP - p-Terphenyl
TPB - 1,1,4,4-Tetraphenylbutadiene

†Ratio of photometer current produced by standard source

‡s = second
m = minute
h = hour

REACTION CONDITIONS: All compounds 5×10^{-3}M in 0.1M K^+tBuO^- in DMSO; sensitizers 10^{-4} unless otherwise noted.

Table 4

CHEMILUMINESCENCE OF MISCELLANEOUS COMPOUNDS

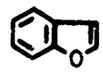
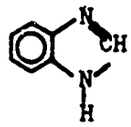
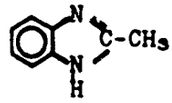
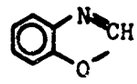
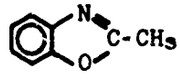
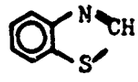
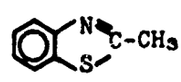
Compound and Structure	Peak O_2 Current Ratio I/I_0	Time to O_2 Peak sec	$T_{1/2}$ of O_2 Peak sec	Figure of Merit
a) Heterocyclic Analogues of Indene				
Benzofuran 	6×10^{-2}	15	35	2.1
Benzimidazole 	7.6×10^{-4}	30	120	9×10^{-2}
2-Methylbenzimidazole 	1.3×10^{-3}	18	72	9.4×10^{-2}
Benzoxazole 	6×10^{-3}	22	72	0.43
2-Methylbenzoxazole 	5×10^{-2}	30	42	0.2
Benzothiazole 	$(2.6 \pm 0.6) 10^{-3}$	15	45 ± 15	0.11
2-Methyl-Benzothiazole 	2×10^{-3}	12	12	24×10^{-3}

Table 4 (continued)

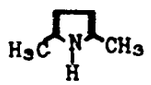
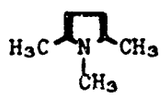
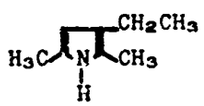
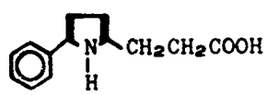
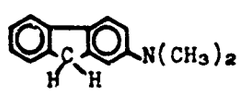
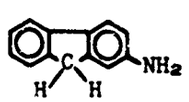
Compound and Structure	Peak O_2 Current Ratio I/I_0	Time to O_2 Peak sec	$T_{1/2}$ of O_2 Peak sec	Figure of Merit
	b) Pyrrole Derivatives			
2,5-Dimethylpyrrole 	1.2×10^{-2}	6	52	0.62
N-Methylpyrrole	8×10^{-3}	15	20	0.16
1,2,5-Trimethylpyrrole 	10^{-3}	10	45	4.5×10^{-2}
2,4-Dimethyl-3-ethyl Pyrrole 	3.2×10^{-3}	60	90	0.29
5-Phenyl-2-pyrrole Propionic acid 	1.6×10^{-2}	8	9	0.14
	c) Fluorene and Fluorenone Derivatives			
2-Dimethylamino fluorene 	$(2.2 \pm 0.7)10^{-2}$	32	5 ± 1	0.11
2-Amino fluorene 	1.9×10^{-2}	26	11	0.21

Table 4 (continued)

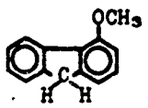
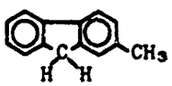
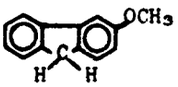
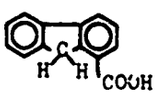
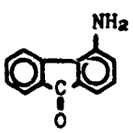
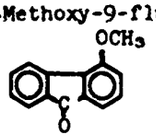
Compound and Structure	Peak O_2 Current Ratio I/I_o	Time to O_2 Peak sec	$T_{1/2}$ of O_2 Peak sec	Figure of Merit
4-Methoxyfluorene 	$(2.5 \pm 0.3)\ 10^{-2}$	17	66 ± 54	1.8
2-Methylfluorene 	8.4×10^{-2}	30	10	0.84
3-Methoxyfluorene 	0.4 ± 0.15	26 ± 10	10 ± 7	4.2 ± 3.6
1-Fluorene-carboxylic Acid 	6×10^{-2}	24	30	1.8
9-Fluorenone-1-carboxylic Acid	3.4×10^{-2}	6	132	4.5
4-Amino-9-Fluorenone 	1.1×10^{-3}	12	24	26×10^{-3}
4-Methoxy-9-fluorenone 	2.2×10^{-3}	24	54	0.119

Table 4 (continued)

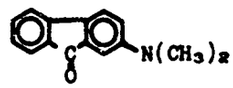
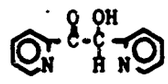
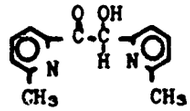
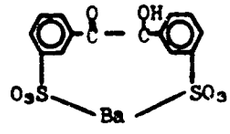
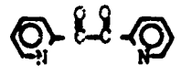
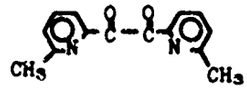
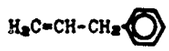
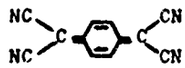
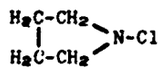
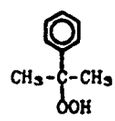
Compound and Structure	Peak O_2 Current Ratio I/I_o	Time to O_2 Peak sec	$T_{1/2}$ of O_2 Peak sec	Figure of Merit
2-Dimethylamino-9-fluorenone 	2.2×10^{-2}	17	24	0.53
d) Acyloins and 1,2-diketones				
α-Pyridoin 	$(2.3 \pm 0.3)10^{-2}$	31 ± 17	3 ± 1	$(6.4 \pm 1.2)10^{-2}$
6,6'-Dimethyl-2,2'-pyridoin 	3.5×10^{-2}	40	2	7×10^{-2}
Barium Benzoin-3,3'-Disulfonate 	$\approx 0.6 \times 10^{-3}$	<5	≈10	$\approx 6 \times 10^{-3}$
2,2'-Pyridyl 	1.4×10^{-2}	12	3	4.2×10^{-2}
6,6'-Dimethyl-2,2'-pyridyl 	1.2×10^{-3}	6	3	3.6×10^{-3}

Table 4 (continued)

Compound and Structure	Peak O_2 Current Ratio I/I_o	Time to O_2 Peak sec	$T_{1/2}$ of O_2 Peak sec	Figure of Merit
e) Compounds Containing an Olefinic Linkage				
Sorbic Acid	1.8×10^{-3}	10	20	39×10^{-3}
Tetracyanoethylene	4.8×10^{-3}	5	10	48×10^{-3}
Allyl benzene 	1.7×10^{-3}	≈10	≈30	5.1×10^{-3}
Tetracyanoquinodimethane (TCNQ) 	2×10^{-4}	<5	--	--
f) Miscellaneous				
N-chlorosuccinimide 	1×10^{-3}	25	120	.120
Cumene Hydroperoxide 	5.4×10^{-3}	10	10	54×10^{-3}
Acenaphthene	1.4×10^{-3}	17	60	8.4×10^{-2}
3-Aminofluoranthene	9.2×10^{-3}	190	84	0.77

APPENDIX V

TOXICITY OF SELECTED COMPOUNDS

No.	Compound	Toxicology	Toxic Hazard Rating
1	Acridine	Strongly irritating to skin and mucous membranes; causes sneezing upon inhaling.	
2.	Acrolein	Strong irritation of skin and exposed mucous; habitual exposure seems to lead to some tolerance; no other chronic ill effects have been reported.	
3	Acrylonitrile	Weakness, light-headedness, headache, nausea, sneezing, abdominal pain, vomiting, loss of consciousness, cessation of respiration (asphyxia) and death.	Maximum allowable concentration is 20 ppm.
4	Benzoin		Acute local: irritant 1 allergen 1 ingestion 1 Acute systemic U Chronic local: allergen 1 Chronic systemic: U
5	Crotonaldehyde	A lachrymating material which is very dangerous to the eyes.	Acute local: irritant 3 allergen 2 ingestion 3 inhalation 3

APPENDIX V (Continued)

No.	Compound	Toxicology	Toxic Hazard Rating
	Crotonaldehyde (continued)		Acute systemic: U Chronic local: allergen 2 Chronic systemic: U
6	Indole		Lethal dosage i.v. in dogs 60 mg/kg
7	Methyl vinyl ketone	Skin irritant and lachrymator.	
8	Skatole		Minimum lethal dosage s.c. in frogs 1.0 g/kg.
9	L-Tryptophan	Medical use: nutrient. Recommended intake for normal adult male 0.5 g/day. Tryptophan and proteins containing Tryptophan are effective in preventing and treating pellagra.	

Note: Toxic rating code: 0 = none
1 = slight
2 = moderate
3 = high
U = unknown

References: 1. "Dangerous Properties of Industrial Materials", Sax, Newton Irving, Reinhold Pub. Co., New York, 1957.

2. "Merck Index of Chemicals and Drugs", Paul G. Stecker (Ed), 7th Ed., Merck Chem. Co., Rahway, New Jersey, 1960.

APPENDIX VI

STRUCTURAL CORRELATIONS AMONG THE BRIGHTER ORGANIC CHEMILUMINESCENT SYSTEMS

Among the more efficient chemiluminescent systems are luminol, lophine, and skatole. The structures of the reacting molecules and their most probable light emitting intermediates are presented in Figure 33.

There are several striking similarities in the type of reaction and nature of the reactants and products. The nature of the oxidation is equivalent to a bond cleavage with the ultimate formation of two carboxyl groups. The products have several features in common:

1. Each system requires a basic reagent. There is at least one acidic amino proton present in each starting material.

2. Oxygen is the common oxidizing agent. Peroxides or hydroperoxides are postulated as chemical intermediates in each case.

3. Two carbonyl groups are formed in the reaction product. At least one is an aryl carbonyl.

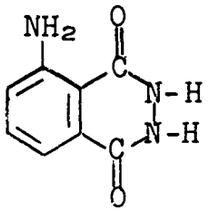
4. An amino group or amino anion is found in each reaction product.

5. A conjugated, fluorescent product is formed in each case.

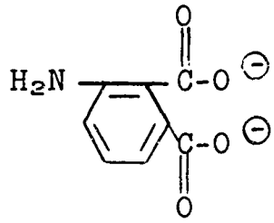
The structural similarities between the products of the skatole and lophine reactions are especially striking.

The amino group or anion may play an important role in determining the emission characteristics of the products.

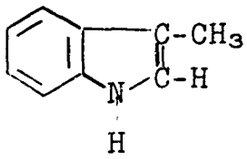
In simple aryl carboxyl compounds (e.g. acetophenone, benzophenone, and benzil) no luminescence is observed in air-saturated liquid solution at room temperatures. This is due to an efficient $S^1 \rightarrow T^1$ carbonyl transition followed by oxygen quenching of the triplet state. The presence of a nitrogen atom may enhance the luminescence by introducing a new electron transition favorable toward light emission. Alternatively, the



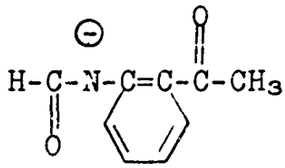
Luminol



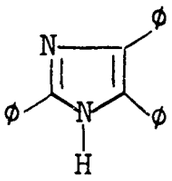
3-Aminophthalate Ion



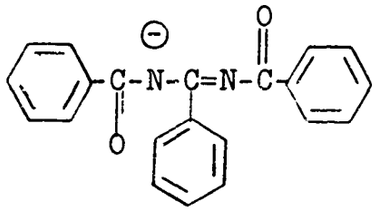
Skatole



Anion of Ortho Formamidoacetophenone



Lophine



Anion of Dibenzoylphenylamidine

Figure 33. Structural Similarities Among Three Efficient Chemiluminescent Systems

nitrogen-containing groups may act as electron-donating substituents, perturbing the transition probability relative to an unsubstituted carbonyl compound.

The similarities among reaction conditions and structures of reactants and products suggest that other common features will be found when the detailed reaction mechanisms are determined.

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1. ORIGINATING ACTIVITY (Corporate author)	2a. REPORT SECURITY CLASSIFICATION
Monsanto Research Corporation Boston Laboratory Everett, Massachusetts 02149	Unclassified
	2b. GROUP

3. REPORT TITLE

CHEMILUMINESCENT SYSTEMS

4. DESCRIPTIVE NOTES (Type of report and inclusive dates)

Final Report: 1 June 1964 - 31 May 1966

5. AUTHOR(S) (Last name, first name, initial)

Driscoll, John S.; Pirog, J.A.: and Berger, A.W.

6. REPORT DATE	7a. TOTAL NO. OF PAGES	7b. NO. OF REFS
November 1966	155	51

8a. CONTRACT OR GRANT NO.	9a. ORIGINATOR'S REPORT NUMBER(S)
Nonr-4511(00)	MRB3009F
b. PROJECT NO. ARPA Order No. 299, Amend. 6	9b. OTHER REPORT NO(S) (Any other numbers that may be assigned this report) none
c. d. Task NR356-464	

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13. ABSTRACT

The objective of this research program was the discovery of bright chemiluminescent reactions suitable for development into illumination and marking devices for field use. Over 175 organic compounds were investigated in the DMSO/t-BuOK/O_2 system in a general screening program. It is concluded from the data that chemiluminescence is a very general phenomenon. Almost every compound studied gave at least weak emission. The indoles were the most promising class of compounds studied. A detailed study of the effect of substituents on indole chemiluminescence was carried out. An investigation of 56 indole derivative showed numerous explainable substituent effects. Skatole (3-methylindole), a natural product, fulfills these conditions and was found to be the brightest system studied. The chemiluminescent emitter in the skatole system was unambiguously identified as the excited singlet state of the anion of orthoformamidoacetophenone (FAP). The agreement between the chemiluminescence spectrum of the skatole system, and the fluorescence emission spectrum of FAP is among the best to be found for any known system. The emitter in the 2,3-dimethylindole system was shown to have a structure analogous to FAP. Possible reaction mechanisms are described.

DD FORM 1 JAN 64 1473

Unclassified

Unclassified

Security Classification

14. KEY WORDS	LINK A		LINK B		LINK C	
	ROLE	WT	ROLE	WT	ROLE	WT
Chemiluminescence Heterogeneous catalysis Indoles Acyloins Exited states Fluorescence Reaction mechanisms Energy transfer						

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